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The effect of supplemental vitamin C on performance, antioxidant capacity, carcass characteristics, and meat quality of steers fed high sulfur finishing diets

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by

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"I can't go back to yesterday, because I was a different person then."

– Lewis Carroll

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"You're off to great places, today is your day;

your mountain is waiting, so get on your way."

–Dr. Seuss

ABSTRACT

The incorporation of ethanol industry co-products, such as dried distillers grains plus solubles, to feedlot diets in the United States may be inadvertently exposing cattle to high amounts of dietary sulfur. High sulfur diets have repeatedly been reported to decrease growth and carcass performance, health, and copper status; however, little to no information is available concerning the implications that high sulfur diets have on the antioxidant capacity or meat quality of cattle. Thus, the subsequent research trials were designed to: 1) examine the impacts of high dietary S on diet digestibility and macro and micro mineral absorption and retention, 2) determine the effect of supplementing a rumen-protected vitamin C during the entire finishing period of steers on growth performance, trace mineral status, antioxidant capacity, carcass characteristics, and meat quality, 3) determine the optimal dose of supplemental vitamin C within a high sulfur diet on growth performance, blood metabolites, carcass characteristics, and meat quality, and 4) identify the influence of timing of vitamin C supplementation during the finishing period on growth performance, plasma vitamin C and glutathione concentrations, and carcass characteristics. Within our first research objective, the consumption of a high sulfur diet for at least 28 days decreased copper, manganese, and zinc retention in steers. These trace minerals are components of antioxidant enzymes, suggesting a lesser availability of these minerals may decrease antioxidant capacity of the animal. Within our second research objective, supplementing vitamin C (10 g per steer per day) to calf-fed steers consuming a high sulfur (0.55%) diet for 149 days prevented a decline in circulating ascorbate throughout the finishing period and increased marbling scores from high Select to low Choice compared to the high sulfur, non-vitamin C supplemented steers. The inclusion of vitamin C to the high sulfur diet prevented the ratio of oxidized-to-reduced

liver glutathione from rising above the oxidative stress threshold of 10%, while a ratio of 28% was observed in the non-supplemented high sulfur steers, indicating some oxidative stress was occurring. In postmortem muscle of these calf-fed steers, the ante-mortem supplementation of vitamin C increased the presence of the fully autolyzed (76-kDa) subunit of calpain-1, an enzyme involved in the tenderization process, and increased the polyunsaturated fatty acid content of the *longissimus thoracis* compared to the un-supplemented high sulfur steers. In yearling steers, increasing the dose of supplemental vitamin C (0, 5, 10 or 20 g per steer per day) in a high sulfur (0.55%) diet linearly decreased dry matter intake, tended to increase feed efficiency, and increased ribeye area. In postmortem muscle, increases in vitamin E and iron and lesser meat lightness values were noted within the vitamin C supplemented treatments compared to the un-supplemented controls, while no differences in calpain-1 autolysis, shear force, or fatty acid profile of the *longissimus thoracis* were observed. Finally, the addition of vitamin C (10 g per steer per day) for the first 56, 90, or 127 days (entire finishing period) of finishing to calf-fed steers consuming a low (0.31%) or high (0.59%) sulfur diet showed limited effects on performance and carcass traits. The findings of our experiments yielded conflicting results of how supplemental vitamin C is impacting finishing steer growth performance, carcass traits, and meat quality; however, these differences may be attributed to individual animal variability or differing genetics of the steers used in these studies. Further research is warranted to better understand the mechanism by which vitamin C supplementation to finishing cattle is influencing circulating ascorbate concentrations, marbling potential, and ribeye area.

CHAPTER 1.

GENERAL INTRODUCTION

Nutritional and economic incentives associated with ethanol co-products, such as dry distillers grains plus solubles (**DDGS**), have encouraged producers to incorporate greater quantities of these co-products in cattle diets as an alternative to corn. Sulfur content of ethanol co-products is often elevated as sulfuric acid is used during the production process and the concentration of nutrients approximately threefold after starch removal, thus the S content of these products can range from 0.3 to greater than 1% (Klopfenstein et al., 2008; Kim et al., 2012). This variation in S content of co-products is a limiting factor for their inclusion to cattle diets, as increasing dietary S can have deleterious effects on the health, performance, trace mineral status, and carcass traits of finishing cattle (Gould, 1998; Spears et al., 2011; Uwituze et al., 2011; Richter et al., 2012). Additionally, S has been implicated as a co-factor in the development of oxidative stress (Truong et al., 2006), which can hinder live animal performance and express residual effects in postmortem muscle. The role of S in the development of oxidative stress may be associated with a lesser availability of essential trace minerals for incorporation into antioxidant enzymes and the depletion of glutathione, an antioxidant protein that is involved in the removal of excess S from the body.

In the body, vitamin C (**VC**) exhibits several roles in cellular metabolism, and is specifically noted for roles in oxidation-reduction reactions, collagen synthesis, and as an enzyme co-factor (Rebouche, 1991). Vitamin C is synthesized in the liver of cattle and has been presumed to adequately meet daily VC requirements, which is why no exogenous requirement for the vitamin has been established by the NRC (1996). Research has identified

that circulating VC decreases throughout the finishing period (Takahashi et al., 1999), and it is possible that under the circumstance of S-induced oxidative stress, the natural production of VC may not sufficiently meet the daily VC requirements of cattle.

Because high S diets have become more common in the United States, methods to cope with the deleterious effects of S on performance and carcass traits are of great interest. This research sought to characterize the impacts that supplementing a rumen-protected VC to finishing steers consuming high S diets would have on steer performance, antioxidant capacity, carcass traits, and meat quality.

Dissertation organization

The following chapter, 2, will provide a detailed review of the literature in regard to S in feedlot cattle diets, development and implications of oxidative stress in the body, and the roles of VC in biological systems. The remaining six chapters present research that has been accepted or submitted to *The Journal of Animal Science* or *Meat Science*. Specifically, chapter 3 examines the influence of high dietary S on diet digestibility and mineral retention of steers. Chapters 4 and 5 contain research conducted on a single set of cattle to specifically address the influence of VC supplementation rate in calf-fed steers fed finishing diets containing varying concentrations of dietary S (0.22, 0.34, and 0.55%), on growth and performance, antioxidant status, carcass characteristics (chapter 4), and meat quality (chapter 5). Similarly, chapters 6 and 7 stem from one set of yearling steers that were used to evaluate four different doses of VC (0, 5, 10, or 20 g $VC \cdot h^{-1} \cdot d^{-1}$) supplemented within 0.55% S finishing diet, on growth performance, blood metabolites, carcass characteristics (chapter 6), and meat quality (chapter 7). The final research chapter, 8, examines the influence of timing

of VC supplementation during the finishing period (the first 0, 56, 90 days, or entire finishing period) to steers consuming a low S (0.25%) or high S (0.55%) diet on performance, circulating VC and glutathione concentations, and carcass characteristics. Finally, this dissertation will conclude with overall research findings and suggestions for future research.

CHAPTER 2.

REVIEW OF THE LITERATURE

Sulfur

Sulfur (**S**) is an essential element for all living species, as S contributes to the production of the S-containing amino acids (methionine and cysteine) and B-vitamins (biotin and thiamin), is a critical component of sulfhydryl bonds found in enzymes and glutathione (**GSH**), and aids in detoxification and protection against pro-oxidants (Suttle, 2010). In addition to the animal's requirements, bacteria (specifically cellulolytic bacteria) in the rumen also require S for adequate growth (Spears et al., 1976). In ruminants, a S-deficient diet may be characterized by decreased appetite and diet digestibility, increased salivation and rumination time, and a reduction in growth and performance. The decrease in the health and performance of cattle may relate to a depression in microbial productivity, as cellulolytic bacteria digest greater quantities of fiber when adequate concentrations of S are present in the rumen environment (Spears et al., 1976).

Sources of Sulfur in Feedlot Cattle Diets

The NRC (1996) recommends a dietary S concentration of 0.15% in cattle diets, while the maximum tolerable level has been based on the percent forage in the diet (NRC, 2005) to avoid the deleterious effects of high S diets on health (polioencephalomalacia; **PEM**) and feedlot performance. In finishing cattle diets containing less than 15% forage, S concentration is recommended to not exceed 0.30% S; while increasing the forage concentration to at least 40% increases the maximum tolerable level for S to 0.50% (NRC,

2005). The minimal requirement, 0.15% S, is often met by the incorporation of common feedstuffs into the diet. However, greater concentrations of S may be introduced to cattle through high sulfate water (greater than 300 mg/L; APHIS, 2000) or greater inclusion rates of ethanol industry co-products, such as distillers grains (wet, dry, or modified), which may contain 0.3 to greater than 1.0% S (Kim et al., 2012).

A survey conducted by APHIS (2000) reported the majority of the United States has water sulfate concentrations below the accepted threshold of less than 300 mg/L. However, 7.7% of water samples (primarily in South Dakota) had a sulfate concentration greater than1000 mg/L. The NRC (2005) indicates water sulfate concentrations should be less than 600 mg/L. Loneragan et al. (2001) evaluated the effects of varying concentrations of sulfate (125, 250, 500, 1000, or 2000 mg/L sulfate) in drinking water on steer (n = 48/treatment) performance during a 113 d finishing trial. Steers consumed approximately 0.18 to 0.40 % S per day from water (as expressed on a percentage of diet DM) plus 0.16% S from the diet. When drinking water sulfate concentrations exceeded 291 mg/L, Loneragan et al. (2001) reported a decrease in dry matter intake (**DMI**), average daily gain (**ADG**), and feed efficiency (**G:F**). Beauchamp (1984) suggested the depression in performance might be related to greater circulating sulfide concentrations that may be contributing to cellular injury. Within the parameters of the Loneragan et al. (2001) study, steers consumed approximately 0.7% S from a combination of high sulfate (2000 mg/L) drinking water and diet, indicating high sulfate water may be especially detrimental during periods of warm ambient temperatures because of an increased water consumption by cattle.

As a means to simulate the effects of Australian range cattle consuming coal mine pit water that is often high in solutes and sulfate, Robertson et al. (1996) supplied low sulfate

"town water" or high sulfate water (2000 mg/L sulfate) to 12 Brahman steers (352 kg body weight; **BW**) for 46 d during a metabolism trial to evaluate DMI, diet digestibility, mineral balance and retention, and blood metabolites. Contrary to Loneragan et al. (2001), Robertson et al. (1996) reported no difference in DMI, organic matter apparent digestibility, mineral retention, or blood metabolites (except Mg) between the two concentrations of sulfate water (Robertson et al., 1996). However, an increase in plasma Mg and the percent mineral excreted as a percent of total nutrients in the drinking water (Ca, Cl, Mg, Na, and S) were observed when steers consumed the high sulfate water.

Distillers grains, produced by the fermentation of corn (or other grains) to yield ethanol and carbon dioxide, are a nutritional feedstuff for livestock. The fermentation process removes the starch from the kernel, thereby concentrating the remaining nutrients (fat, protein, and fiber) by approximately three fold (Klopfenstein et al., 2008). An inclusion of 10 to 15% distillers grains to cattle diets is sufficient to meet protein requirements, while inclusions greater than 15% also provide energy to cattle diets. The feeding value of dried distillers grains plus solubles (**DDGS**; 20% inclusion) is 126% the feeding value of dry rolled corn, and wet distillers grains (**WDGS**; less than 15% inclusion) is estimated to be approximately 160% the value of dry rolled corn (Klopfenstein et al., 2007). In addition to concentrating other nutrients, distillers grains often contain elevated S concentrations, ranging from 0.3 to greater than 1.0%, as sulfuric acid is used to control pH during fermentation and during the cleaning process (Klopfenstein et al., 2008; Kim et al., 2012). Economic and nutritional incentives are encouraging greater inclusions of distillers grains to finishing cattle diets, and thereby increasing the concentrations of dietary S fed to cattle.

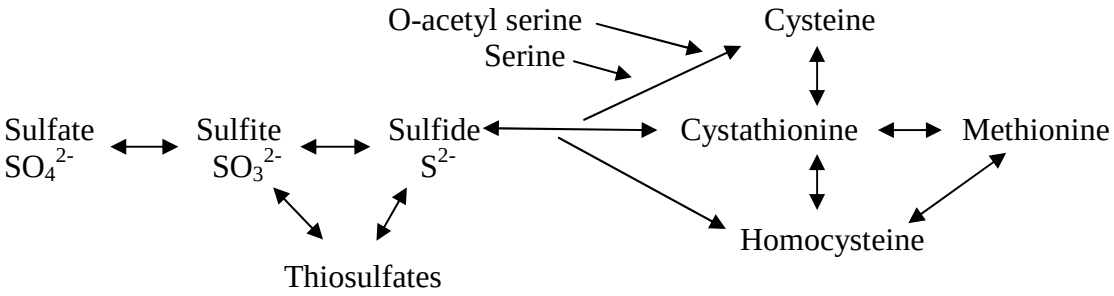


Figure 1. Sulfate is reduced to sulfide by rumen bacteria and is incorporated into proteins.

Rumen Sulfate Reducing Bacteria

A plethora of substrates are available in the rumen to sustain a great diversity of microbes. Sulfate-reducing bacteria (**SRB**), as the name implies, are responsible for the reduction of sulfate to sulfide in the rumen, via assimilation or dissimilation (Cummings et al., 1995a). The assimilatory pathway reduces a sulfate ion from an oxidized to fully reduced status (Postgate, 1979), for the incorporation of S into S-containing amino acids and B-vitamins (Kandylis, 1984; Suttle, 2010). Alternately, under the anaerobic conditions of the rumen, SRB can use inorganic S as a terminal electron acceptor for the production of ATP to support growth via the dissimilatory pathway (or sulfate respiration; Rees, 1973). The model of sulfide production by SRB, identified by Rees (1973), follows a linear chain of events in which sulfate entering the cell, likely in conjunction with hydrogen (Cypionka, 1989), is converted to adenoside phosphosulfate and undergoes two reductions, first to sulfite and then to sulfide. The production of sulfide in the chain of reactions is the terminal reduction facilitated by dissimilatory sulfite reductase (Rees, 1973). The reduction of sulfate by SRB is catalyzed by dissimilatory sulfate reductase, and the capacity of SRB to reduce sulfate relates to the quantity of the *dsrA* gene transcript present in SRB cells (Strattan, 2010).

Sulfate-reducing bacteria have adapted to thrive in nearly all types of environments, including the rumen, marine sediments, deep-sea hydrothermal vents, and oil fields (Barton and Fauque, 2009). While these species are able to survive in rumen-like environments, their contribution to the rumen bacterial population (10^{10} to 10^{11} cells/g of rumen contents; Yokoyama and Johnson, 1988) as a whole is quite low, as *Desulfovibrio* and *Desulfotomaculum* species are estimated to account for approximately 10^{8} organisms/mL and 100 organisms/mL of rumen contents (Howard and Hungate, 1976).

Sulfate-reducing bacteria, typically of the *Desulfovibrio* species, perform maximal sulfate reduction in the presence of lactate, alanine, formate, and pyruvate while no growth was noted when incubated with acetate (Coleman, 1960; Howard and Hungate, 1976). The substrates are in greater proportion when cattle consume diets with a greater proportion of fermentable carbohydrates (starch) and less forage, thus finishing cattle consuming S are a more optimal host for SRB. Consequently, increasing starch results in a decrease in pH (increasing hydrogen), which may encourage the reduction of sulfate by SRB. Furthermore, the decreased pH (below 7.04, the second pKa value for hydrogen sulfide) resulting from starch fermentation promotes the formation of hydrogen sulfide (**H_2S**) in the rumen from the combination of sulfide (from SRB) and hydrogen (Kandylis, 1984; Kung et al., 1998; Schoonmaker and Beitz, 2012). The actions of SRB are believed to be critical in the etiology of S toxicity in ruminants.

Sulfur Metabolism and Toxicity

In the rumen, Bray (1969) reported sulfide was more rapidly and directly absorbed across the rumen wall, as evident by an increase in blood sulfide concentration, while the

movement of sulfate across the rumen is less likely. Sulfide absorbed across the rumen wall is transported via the portal blood to the liver for further metabolism to sulfate, and excretion by way of urine (Kandylis, 1984). Hydrogen sulfide, on the other hand, gains entry to the general circulation via alveolar diffusion (Gould, 1998). This occurs during eructation, as Dougherty and Cook (1962) indicated that 70 to 80% of the eructated H_2S might be inhaled by the animal. Once in general circulation, H_2S is more likely to have negative impacts on neurological and cardiac function by inhibiting ATP production (Dougherty et al., 1965; Kandylis, 1984; Hall, 2012), as H_2S interacts with the extremities prior to further metabolism in the liver. The removal of S from the body has been noted to occur by binding oxygenated hemoglobin in the blood (forming sulfhemoglobin, further discussed later in the current section) and removed when red-blood cells are recycled, or by conjugation with disulfide containing proteins, such as glutathione (**GSH**; Beauchamp, 1984; Kandylis, 1984).

Entry of S, as sulfide or H_2S, into the blood stream can result in the irreversible formation of sulfhemoglobin, a product of the covalent bonding between H_2S and the heme-Fe (Fe^{2+}) in hemoglobin. Sulfhemoglobin decreases the oxygen-carrying capacity of the blood, as the Fe in sulfhemoglobin no longer possesses the ability to bind and transport oxygen (Keilin, 1933). Triapirux et al. (2008) reported sulfhemoglobin toxicity occurs when sulfhemoglobin concentrations exceed 1% of the total hemoglobin. The resolution of sulfhemoglobin in the blood occurs only when red blood cells are recycled, as the formation of sulfhemoglobin is permanent. Drewnoski et al. (2012) reported steers consuming a high S (0.68%, n = 8) diet had a greater concentration of sulfhemoglobin compared to steers consuming a control diet (low S, 0.24%; n = 8) after 10 d of consuming a high S diet, and remained greater than the control steers throughout the 28 d study.

While H_2S is an established toxic gas in humans, it has also been shown to have important roles in the regulation of neuron activity and muscle relaxation, specifically by interacting with ATP-sensitive potassium channels, cytochrome *c* oxidase, myoglobin, and hemoglobin (Pietri et al., 2011). These components are interlinked through their roles in ATP production and oxygen transport, as the interaction of H_2S with cytochrome *c* oxidase induces modifications to the copper (**Cu**)-center of the enzyme thus inhibiting its activity in the electron transport chain for ATP production. As mentioned previously, hemoglobin and myoglobin are responsible for the delivery of oxygen to tissues and mitochondria during ATP production and the competitive binding of H_2S to either hemoglobin and myoglobin can decrease the oxygen-carrying capacity and subsequently, ATP production (Pietri et al., 2011). In the cell, when ATP production is adequate the ATP-sensitive potassium channel is blocked from being open, thus enabling the cell to depolarize (influx of calcium, **Ca**) for numerous functions (depending on the cell type) including muscle contraction, nerve signal transduction, or the release of enzyme granules into the blood or lumen. Alternately, this process is blocked when ATP production is insufficient. Within the ruminant, Dougherty et al. (1965) postulated that similar to humans, a negative effect of H_2S on smooth muscle contractions might be occurring in the rumen, thus limiting or hindering rumen motility and subsequently reducing DMI of cattle consuming high concentrations of S.

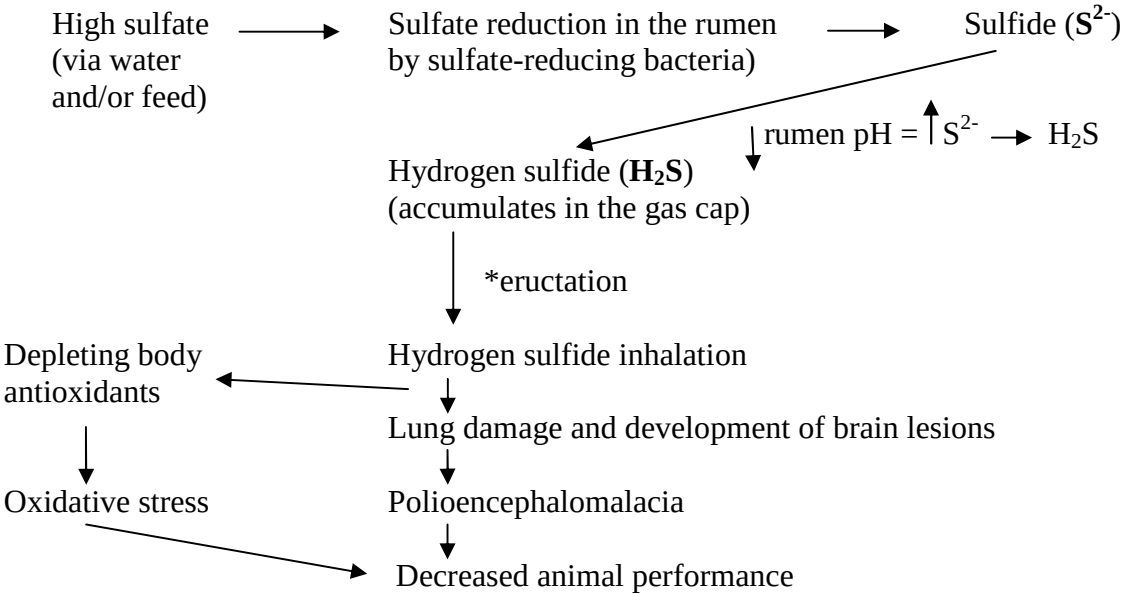
Sulfur-induced PEM is a clinical manifestation of S toxicity (Figure 2), and is characterized by head pressing, staggering, blindness, and brain lesions (Dill, 1986). Symptoms of PEM are associated with the inhalation of eructated H_2S, which enables the gas to gain access to the blood stream by alveolar diffusion and exert negative effects within the brain (Gould, 1998). Cattle and lambs consuming high S diets experience a lag time of about

10 to 35 d before maximal H_2S concentrations are produced in the rumen, which may indicate that SRB require an adaptation period (Cummings et al., 1995; Loneragan et al., 1997; Drewnoski et al., 2012; Loerch et al., 2012; Richter et al., 2012). This peak in H_2S production within 35 d of consuming a high S diet indicates that cattle are at the greatest risk of developing S toxicity early in the feeding period.

While the reason for the observed lag time prior to maximal H_2S production by SRB is unknown, some contributing factors may include rumen pH or the availability of the S source. As aforementioned, a rumen pH below 7.04 supports the formation of H_2S (Kung et al., 1998; Schoonmaker and Beitz, 2012). A negative relationship has been identified between rumen pH and H_2S production (Morine et al., 2012; Morrow et al., 2013). Because typical feedlot diets often have greater inclusion of rapidly fermentable carbohydrates and lesser amounts of roughage, rumen pH is unlikely to be above 7.04 (Kung et al, 1998), thus increasing the risk of developing PEM in feedlot cattle.

Inorganic sources of S are presumed to be 100% available in the rumen for reduction by SRB, while the S associated with the amino acids in feedstuffs must first be freed from the protein through proteolysis prior to use by SRB for the production of sulfide. Sarturi et al. (2013) evaluated this concept by comparing the effects of differing sources of S on H_2S production in five fistulated beef steers. The following S sources were balanced to provide similar adjusted ruminal protein S, but at different dietary S concentrations: no additional S control (0.21% S), ammonium sulfate (inorganic source; 0.36% S), corn gluten meal (organic source, 2 concentrations; low, 0.30% S and high, 0.45% S), and wet distillers grains (0.50% S). Hydrogen sulfide production was greatest in cattle fed the inorganic (1,704 to 2,045 μg/L; 1.70 to 2.04 g/m^3) and wet distillers grains (1,533 to 2,045 μg/L; 1.53 to 2.04 g/m^3)

treatments compared to the control (102 to 239 μg/L; 0.10 to 0.24 g/m^3) and organic S treatments (170 to 852 μg/L; 0.17 to 0.85 g/m^3), as the inorganic and wet distillers provided more ruminally available S (0.30 and 0.32%, respectively) than the organic source (0.17 or 0.21%, low and high, respectively). These authors indicated that the availability of S source in the rumen explains 65% of the variation in H_2S production by cattle, while S intake and rumen pH accounted for 29% and 12% of the variation, respectively. It is important to note that no differences in DMI by steers were noted in this study.



Adapted from Kung et al., 1998

Figure 2. Influence of dietary sulfate on the development of sulfur toxicity in ruminants.

High Sulfur Diets and Cattle Performance and Meat Quality

Improvements in feedlot performance, specifically ADG and G:F, have been observed with moderate inclusions (10 to 30%) of distillers grains in feedlot diets when compared to traditional corn-based diets (Buckner et al., 2007; Klopfenstein et al., 2007). The nutrient

profile of DDGS from 10 ethanol plants in Minnesota and South Dakota were determined every two months during the years 1997 to 1999 (totaling 118 DDGS samples; Spiehs et al., 2002). These authors reported a great variation between and within plants among mineral content; specifically the S content ranged from 0.33 to 0.74% with the variation coefficient ranging from 6.4 to 43%. Similarly, Kim et al. (2012) reported the S concentration of DDGS varied from 0.3 to greater than 1%. Because of this variation, S content remains a limiting factor for the inclusion of distillers grains in cattle diets, as high S diets have been shown to negatively affect feedlot performance and health of cattle (Gibson et al., 1988; Zinn et al., 1997; Zinn et al., 1999; Buckner et al., 2007; Uwituze et al., 2011; Richter et al., 2012).

The source of S in cattle diets appears to differentially affect carcass characteristics of finishing cattle when S is increased in the diet (Table 1). Researchers evaluating dietary S concentrations between 0.15 and 0.4% S on cattle performance have observed varying results in regards to the impact of S on DMI, ADG, and G:F. Gibson et al. (1988) evaluated the influence of sulfur dioxide-treated high moisture barley in 64 Hereford and Hereford-cross steers (391 kg BW) during a 96 d finishing trial, in which diets contained 0.17 or 0.46% dietary S (10% roughage). These authors reported cattle consuming the sulfur dioxide-treated diets (0.46% S) had a lesser final BW, ADG, DMI, G:F, and hot carcass weight (**HCW**) compared to cattle receiving non-sulfur dioxide-treated barley diets (0.17% S).

Zinn et al. (1997) evaluated growth performance of heifers fed a corn-based finishing diet with 0.15, 0.20, and 0.25% achieved by the inclusion of ammonium sulfate, for approximately 76 d. The authors reported no differences in heifer performance when consuming either 0.15 or 0.20% S; however, ADG, DMI, final BW, HCW, and rib-eye area (**REA**) decreased as the S inclusion increased from 0.20% to 0.25% (Table 1). Alternately,

another study by the same authors (Zinn et al., 1999) evaluated performance of growing calves (215 ± 10 kg) consuming 0.17, 0.22, and 0.27% S, achieved by addition of ammonium sulfate to a steam-rolled wheat-based diet, for approximately 84 d. No differences in final BW or ADG were detected as dietary S increased; however, G:F linearly decreased as S inclusion increased.

Analogously, Spears et al. (2011) investigated the influence of 0, 0.15, or 0.30% supplemental S from ammonium sulfate on performance and carcass characteristics of 114 steers during the growing (84 d) and 109 d finishing phase. The growing and finishing diets were analyzed to contain 0.13, 0.31, and 0.46% S. Within the growing phase, a dietary S concentration exceeding 0.31% decreased final BW, ADG, and G:F. During the finishing period, the steers consuming the 0.46% S diet continued to have poorer performance when compared to the lesser S diets (0.13 and 0.31% S). The depression in live performance by the 0.46% S steers was also reflected at slaughter with decreased HCW, REA, quality grade, and yield grade when compared to the control and 0.3% S steers (Table 1).

Increasing S concentration in the drinking water has similar negative effects on carcass characteristics, as demonstrated by Loneragan et al. (2001), when water sulfate concentration was increased from 136 to 2,360 mg/L (equivalent to 0.18 to 0.40% S as a percent of the diet DM) for steers (n= 240; 48/treatment; 304 kg BW) over a 114 d finishing period. As the water sulfate concentration increased, these authors noted a linear decrease in steer HCW, dressing percentage, and yield grade, a quadratic effect of sulfate on 12^{th} rib back fat, and no difference in marbling scores (Table 1).

In recent years, the introduction of high S diets to feedlot cattle is likely attributed to greater inclusion of rates of DDGS to the diet, as the S content of DDGS can range from 0.3

to greater than 1% (Kim et al., 2012). Buckner et al. (2007) evaluated cattle performance across inclusion rates of DDGS ranging from 0 to 50%. These authors reported 6 out of 40 steers consuming the 50% DDGS inclusion (0.6% S; 7.5% roughage) either died or displayed symptoms of S toxicity (PEM) within the first 22 d on study. These adverse effects in the 50% DDGS inclusion may be related to the amount of roughage in the diet, which at 7.5% may have been inadequate to maintain a higher rumen pH. As mentioned previously pH below 7.04 increases the formation of H_2S. However, in the other treatments, no adverse effect of S concentration or DDGS inclusion was observed. Richter et al. (2012) reported yearling steers consuming a 0.6% S diet (8% roughage), achieved by the inclusion of sodium sulfate, for 95 d displayed lesser ADG and HCW, but not DMI or G:F (Table 1), in comparison to the steers finished on a 0.3% S diet. Uwituze et al. (2011) evaluated the rumen digestibility and fermentation of cattle consuming high S diets, 0.42 and 0.65% S, achieved by the inclusion of high S DDGS. In this study, steers consuming 0.65% S had lesser DMI and ruminal production of volatile fatty acids, specifically propionate, compared to steers consuming 0.42% S.

Depenbusch et al. (2009) utilized 356 crossbred heifers to test six inclusion rates of DDGS (0, 15, 30, 45, 60, and 75%) within a steam-flaked corn-based diet on growth performance, carcass traits, and meat quality. A linear decrease in DMI, ADG, G:F, final BW, HCW, and 12^{th} rib back fat occurred as DDGS inclusion rate in the diet increased from 0 to 75% (estimated S range of 0.19 to 0.46%; NRC, 1996). Gunn et al. (2009) used Angus-cross steers (n = 15 steers/treatment) to evaluate the impact of 0 or 50% inclusion of DDGS to a dry-rolled corn-based diet (~0.22 and 0.42% S; NRC, 1996) on performance, carcass characteristics, and meat quality. Dry matter intake was not different between the two

treatments, but ADG, G:F, final BW, HCW were lesser in the steers consuming the 50% DDGS diet compared to steers fed the 0% DDGS diet. Variable data are available concerning the impact of S on marbling score. Gunn et al. (2009) and Depenbusch et al. (2009) reported a decrease in marbling score of steers and heifers, respectively, when cattle consumed diets containing greater than 50% DDGS (estimated 0.42 to 0.46% S, based on calculated values; NRC, 1996) compared to a 0% DDGS inclusion (low S, 0.22% S, estimated values; NRC, 1996). Because steers in the Gunn et al. (2009) trial had reached a common back fat thickness, thus limiting differences due to a dissimilar degree of finishing, the effects of DDGS and S on marbling score are more clear compared to Depenbusch et al. (2009), in which steers were not of a similar back fat thickness. Alternately, Koger et al. (2010) did not observe a difference in marbling scores when 240 Angus-cross steers (8 steers/pen, 15 pens/treatment) consumed a 40% DDGS inclusion to dry rolled-corn based diet (estimated 0.51% S; NRC, 1996) compared to 0% DDGS inclusion controls (estimated 0.18% S; NRC, 1996), interestingly back fat thickness was greater in when DDGS was included in the diet compared to control. While the impact of dietary S on marbling score varies, one factor that may need greater consideration is the number of days cattle are on feed. Because high S diets can negatively impact intake and gain, cattle finished on high S diets may require additional days on feed to reach a similar body composition.

While performance and carcass characteristics from cattle consuming differing inclusions of distillers grains have been extensively evaluated (Klopfenstein et al., 2008), the S content of the diet and the subsequent impacts on meat quality have thus far been overlooked. High S concentrations in the body have been identified as a potentially causative agent in the development of oxidative stress, as evident by a depletion of glutathione (Truong

et al, 2006), which may contribute to an oxidative environment postmortem. It is well established that a postmortem oxidative environment interferes with proteolysis thus hindering the tenderization process (Guttmann and Johnson, 1998; Lametsch et al., 2008; Rowe et al., 2004b). Please refer to the section "Oxidative stress and live animal performance and meat quality" for more details regarding oxidation and proteolysis.

It has been reported by others that Warner-Bratzler shear force values of steaks from cattle consuming diets estimated to contain 0.19 to 0.46% S (estimated values; NRC, 1996) are not affected (Depenbusch et al., 2009; Gunn et al., 2009). Increasing the S content in the diet may decrease the antioxidant capacity that is necessary to sustain the color shelf-life of meat products, specifically resulting in a deterioration of color and lipid stability for greater meat surface discoloration and production of "off-flavors" (Renerre et al., 1996; Wood et al., 2004). Depenbusch et al. (2009) observed that as DDGS inclusion in heifer diets increased from 15 to 75% (estimated range of 0.19 to 0.46% S; NRC, 1996) steaks were progressively less red on d 7 of retail display and contained a greater percent of the brown-pigmented metmyoglobin, which is formed when the heme-Fe^{2+} of myoglobin is oxidized to Fe^{3+}. Because consumer selection of beef retail products is based primarily on color, increasing the quantity of metmyoglobin (brown-pigmentation) in these products decreases the appeal to consumers (Grunert, 1997). While Grunert (1997) did not evaluate the antioxidant capacity, no differences were observed in thiobarbituric acid (**TBA**) content of steaks. Similarly, Gunn et al. (2009) noted no difference in TBA values of steaks collected from steers consuming 0.21 to 0.46% S (based on 1996 NRC values). Alternately, Koger et al. (2010) observed an increase in TBA content of the *longissimus* muscle collected from steers consuming a 40% DDGS diet (estimated 0.51% S; NRC, 1996) compared to steers consuming a 20% DDGS

diet (estimated 0.31% S; NRC, 1996). Due to the variability in content and animal response to S and DDGS inclusion, more research is needed to determine the impacts of dietary S on meat quality.

Table 1. Impact of dietary sulfur on feedlot cattle performance

Reference	Low and High S Concentrations	Diet Type	Source of S	Cattle Type	Findings
Richter et al., 2012	0.3 and 0.6% S	Concentrate (40% DDGS)	Sodium sulfate	Yearling steers	↓ADG: (10.9%; -0.17 kg/d) ↓HCW (4.8%; -17 kg)
Uwituze et al., 2011	0.42 & 0.65%	Concentrate, SFC or DRC (50%); ~30% DDGS	Sulfuric acid	Yearling steers	↓Final BW (4.5%; -22.5 kg) ↓DMI (19.5%; -0.9 kg/d) ↓HCW (6%; -18 kg)
Zinn et al., 1997	0.15, 0.2, & 0.25% S	Concentrate (73.3% flaked corn)	Ammonium sulfate	Yearling heifers	↓Final BW (5.2%; -23.5 kg) ↓DMI (9.8%; -0.68 kg/d) ↓HCW (4.7%; -13.8 kg) ↓REA (8.5%; -6.7 cm^2)
Spears et al., 2011	0.13, 0.32, & 0.46% S	Concentrate (85% ground corn)	Ammonium sulfate	Steers	↓Final BW (7.6%; -37.5 kg) ↓DMI (23.5%; -1.73 kg/d) ↓HCW (8.8%; -25.2 kg) ↓REA (5.3%; -4 cm^2)
Loneragan et al., 2001	136, 291, 583, 1,219, 2,360 mg sulfate/L Diet equivalent: 0.18, 0.19, 0.22, 0.29, & 40% S	Concentrate (77.4% Steam flaked corn)	Sulfate (water)	Steers	↓Final BW (-0.45 kg) ↓HCW (-0.38 kg) ↑REA (+0.22 cm^2)

Sulfur Interactions with Other Minerals

Dietary S has been identified as a causative agent in the disruption of metabolism of some minerals in the ruminant, primarily by antagonizing absorption via formation of insoluble complexes or competition for transporters (Shrift, 1954; Underwood and Suttle, 1991). Much of the research available concerning S antagonism has been with Cu and selenium (**Se**; Shrift, 1954; Ganther and Bauman, 1962; Underwood and Suttle, 1991; van Ryssen et al., 1998, Ivancic and Weiss, 2001). While less examined, others have identified interactions of S with Ca, magnesium (**Mg**), and zinc (**Zn**; Shrift, 1954; Spears et al., 1985; Qi et al., 1993; Felix et al., 2012).

The formation of Cu-sulfide is a well-studied antagonism by S (Underwood and Suttle, 1991; Spears, 2003). Furthermore, the introduction of molybdenum (**Mo**) to the rumen or circulation results in the scavenging of Cu by the S-Mo compound thiomolybdate, which may exist in differing states (di-, tri-, or tetra-thiomolybdate) depending on the number of S bound to the Mo (Dick et al., 1975; Suttle, 1991), thus further diminishing the Cu available for utilization in biochemical processes (Underwood and Suttle, 1991). Because less Cu is absorbed as a result of the formation of Cu-sulfide or the complex formed between Cu, S, and Mo in the intestinal tract, a greater loss of Cu is observed in feces of cattle consuming a high S diet (Underwood and Suttle, 1991). The inability of the animal to utilize dietary Cu may lead to a deficiency, classically expressed by poor growth, reduced coat quality (pigmentation), emaciation, and diarrhea (Underwood and Suttle, 1991).

The interaction between S and Cu has been examined in cattle, sheep, and goats with varying responses. Steers (n = 114; 253 kg BW) consuming 0.12, 0.32, and 0.51% S in a growing diet (87% corn silage) for 84 d did not display differences in plasma Cu or Zn

concentrations (Spears et al., 2011). However, increasing the proportion of concentrate in the diet to 86% ground corn and maintaining the S concentrations of the growing diet (0.13, 0.32, and 0.46% S) during the finishing period (109 d) resulted in decreased plasma Cu, but not Zn. Increasing the S content of the diet from 0.13 to 0.46% corresponded to a 134 mg Cu/kg DM decrease in liver Cu concentrations on d 101 of the finishing study. Additionally, glutathione peroxidase activity, a Se-dependent enzyme, was decreased as dietary S increased. Similarly, Richter et al. (2012) reported no difference in plasma or liver Cu or Zn status when steers were grown on bromegrass pasture for 37 d consuming either a low S (0.3%) or high S (0.45%) prior to entry into the feedlot. However, consumption of a high S (0.6%) feedlot diet (9% roughage) for 87 d decreased both plasma and liver Cu concentrations of steers compared to the steers consuming a low S (0.3%) diet.

Bremner and Young (1978) observed an increase in plasma Cu concentrations and a decrease in liver Cu concentration in ewe lambs (n=12; 22 kg BW) supplemented with 0 or 5 g sulfate/kg (from sodium sulfate) and 25 mg Mo/kg for 30 weeks during finishing; however, Cu concentration were greatest in the kidney of the sulfate and Mo supplemented group. Similarly, wether lambs (n = 24) consuming increasing amounts of DDGS in a finishing diet (0 to 60% DDGS, 0.13 to 0.35 % S) for 20 d had decreased liver Cu and Zn (Felix et al., 2012). Qi et al. (1993) reported no difference in plasma Cu or Zn concentrations when Angora goats (n = 8; 47.8 kg) were used in a 4 × 4 Latin Square experiment to evaluate the impact of dietary S (0.16, 0.23, 0.29, or 0.34% S) on mineral status (20% forage diet). While circulating mineral was not altered, net retention of Cu was greater in the goats fed 0.29 and 0.34% S diets compared to the goats consuming 0.16 and 0.23% S diets (Qi et al., 1993).

While the antagonism between Cu, Zn, and S is primarily related to physical binding of the compounds together (as Cu-sulfide or Zn-sulfide), the relationship between S and Se relates to competitive transportation across the small intestine (Shrift, 1954). The concentration of one element will dictate the absorption of the other. Because limited research data are available concerning the competition in mammalian species, McConnell and Cho (1965) evaluated the interaction of S and Se in the intestine of hamsters, using the everted intestinal-sac technique. Briefly, this method is a quick test of intestinal transport of a compound, in which a section of intestine is everted, filled with the compound of interest, placed in tube with oxygenated media buffer and agitated, and after a set period of time the media buffer is then analyzed for the compound. In contrast to data from plants and bacteria, the transmucosal movement of selenite was not altered when molar concentrations of S (as sulfite) in the medium exceeded the concentrations of Se (as selenite) by 1.7 or 3.3 times. Ganther and Bauman (1962) observed an increase in urinary Se excretion when sodium sulfate was included at 1% of the diet of young male rats (127 to 153 g BW) compared to rats consuming a diet with no added sodium sulfate. Interestingly, sulfate inclusion to the diet had a greater impact on radiolabeled urinary Se excretion when the Se source was injected selenate (46.1% of labeled Se was excreted in urine) compared to injected selenite (21.4% labeled Se excreted in the urine). Authors suggested this discrepancy in sources of Se excreted might be related to an antagonism between sulfate and selenate, due to their similar structures.

The interaction between Cu, Se, and S in finishing sheep (n = 41; 23 kg BW) was evaluated by van Ryssen et al. (1998) using a 3 (0.35, 0.88, and 1.34 mg Se added as sodium selenite/kg feed) □ 2 (6.7 and 17 mg Cu added as copper sulfate/kg feed) □ 2 (2.15 and 3.97

g S added as sodium sulfate/kg feed) factorial design. Increasing the concentration of S from 2.15 to 3.97 g S/kg feed decreased liver Cu within both the low Cu treatment (6.7 mg Cu/kg feed; 228 and 173 mg Cu/kg DM, respectively) and high Cu treatment (17 mg Cu/kg feed; 952 and 473 mg Cu/kg DM, respectively), and within the low Se treatment (0.35 mg Se/kg feed; 1.85 and 1.54 mg Se/kg DM, respectively), medium Se treatment (0.88 mg Se/kg feed; 3.42 and 2.29 mg Se/kg DM, respectively), and high Se (4.61 and 3.47 mg Se/kg DM, respectively). Additionally, increasing S concentration to the diet decreased the presence of Se in rumen bacteria, which authors suggest may reflect a lesser incorporation of Se in place of S into amino acids. Similarly, Ivancic and Weiss (2001) supplemented lactating dairy cows (n = 30) with 0.1 or 0.3 mg Se/kg DM (Na selenate) within a 0.2, 0.4, or 0.7% S (Ca and Mg sulfate) diet for 112 d. Increasing the concentration of S in the diet linearly decreased the apparent digestibility of Se (low Se: 38.6, 29.0, and 27.2, and high Se: 46.9, 37.2, and 33.0%, respectively), absorbed Se (low Se: 1.04, 0.80, and 0.70 mg Se/d, and high Se: 2.24, 1.69, 1.56 mg Se/d, respectively), and retained Se (low Se: 0.04, -0.08, and -0.11 mg Se/d, and high Se: 0.46, 0.04, and -0.02 mg Se/d, respectively). These authors suggest the decrease in Se digestibility, absorption, and retention caused by dietary S concentration may be associated with alterations in Se metabolism in the rumen, as the reducing environment of the rumen can reduce Se to other less available forms, thereby limiting Se utilization and incorporation into the host or microbial populations (van Ryssen et al., 1998).

Minerals play an essential role in immunity, antioxidant capacity, and growth; therefore, additional thought should be given to the recommended trace mineral concentrations of cattle consuming a high S diet. The practice of increasing supplemental mineral concentrations 1.5 to 2 times the normally recommended values during times of

stress, NRC (1996), may have some credence in high S diets, as decreased intakes caused by S and dietary mineral antagonisms with S may limit the quantity of mineral being absorbed and available for incorporation into biological processes.

Sulfur Summary

After reviewing the literature regarding S and cattle, it is clear that dietary S, whether introduced via high sulfate water, inorganic sources, or by co-product inclusion, can have detrimental impacts on feedlot cattle growth performance, health, trace mineral status, and carcass characteristics. However, given the currently available information concerning S, the aspect that has received little attention is the role of S in the development of oxidative stress (please refer to the following section “Oxidative Stress” for additional information). Because dietary S may be decreasing mineral consumption (result of decrease DMI) and limitations on absorption/availability of mineral (resulting from gastrointestinal antagonisms), cattle consuming a high S diet may be at greater risk of developing trace mineral deficiencies and also oxidative stress, as trace minerals are essential components of several antioxidant enzymes. As trace minerals are a necessary for many processes in the body, the use chelated (organic) or injectable trace minerals may be viable options to bypass rumen antagonisms.

Additionally, as far as this author is aware, virtually no information is available concerning the impact these elevated S diets may be having on meat quality of feedlot cattle, specifically in relation to protein degradation (and/or tenderness), color and lipid stability, and fatty acid profile of beef. Researchers that have investigated meat quality aspects from cattle consuming differing inclusions of co-products (and presumably increased dietary S concentrations) commonly neglect to report the dietary S and lipid concentrations that

comprise the co-product(s) used or overall S content of the diet, thus making comparisons between research trials difficult.

Oxidative Stress

Oxidative stress has been defined as an imbalance between pro-oxidants and anti-oxidants in the body, which may result in a reduction of growth and performance in livestock and can express residual effects on carcass characteristics and meat quality. Oxidative damage may result in the alteration of macro-molecules, increase apoptosis, and overall structural damage (Lykkesfeldt and Svendsen, 2007).

The use of molecular oxygen as a terminal electron acceptor for the production of ATP is essential in all forms life that depend on aerobic metabolism. A side effect of reliance on oxygen is the production of free radicals, superoxide ions, and reactive oxygen species (**ROS**). These compounds are formed when misdirected electrons join with oxygen, most commonly occurring during the production of ATP via the electron transport chain. Reactive oxygen species contain one or two groups of unpaired electrons and are constantly seeking to restore balance, which increases their affinity for electrons (Berger, 2005). These electrons are often acquired from neighboring molecules or compounds, such as unsaturated fatty acids. During times of increased oxidative metabolism, such as stress, infection, injury, pregnancy, or during compound detoxification, the production of ROS is greatly increased (Nockels, 1996; Sordillo and Aitken, 2009). During an immune challenge, neutrophils generate large quantities of ROS to be released in an oxidative burst to destroy pathogens. However, neither the neutrophils nor “self cells” are protected against this burst and may result in an even greater production of ROS due to tissue injury.

In the presence of free transition metals (specifically Cu and Fe), hydrogen peroxide can form a more severe radical, the hydroxyl radical, via the Fenton reaction. In this reaction, the interaction of Fe^{2+} (or Cu^{1+}) and hydrogen peroxide results in the oxidation of the trace mineral (to Fe^{3+}; or Cu^{2+}) and the formation of hydroxyl radical (Prousek, 2007). The hydroxyl radical is the most potent and reactive radical in biological systems, and has the ability to remove electrons from nearly every organic and inorganic compound (Kohen and Nyska, 2002). The compounds of greatest concern are DNA (base-pair modifications and strand breakage), lipids (peroxidation, altering membrane structure which may compromise the cell integrity), and proteins (carbonylation, aggregation, tagging for degradation, or inactivation of enzymes; Kohen and Nyska, 2002; Rowe et al., 2004*a,b*; Huff-Lonergan and Lonergan, 2005; Kim et al., 2010; Pickering et al., 2013).

Sulfur and Oxidative Stress

Truong et al. (2006) proposed the mode of action by which H_2S results in cytotoxicity and cell death is the result of the formation of ROS, specifically the superoxide ion, hydroxyl radical, and hydrogen peroxide, and reactive sulfur species (**RSS;** Giles et al., 2001; Cooper and Brown, 2008), by the inhibition of cytochrome *c* oxidase and the release of free Fe (Fe^{2+}) from the Fe storage protein, ferritin (Figure 3). Three molecules of H_2S can bind to one cytochrome *c* oxidase, specifically the Fe and Cu cofactors of the enzyme, and this is thought to be a causative action in the development of oxidative stress in animals consuming a high S diet (Schoomaker and Beitz, 2012). Giles et al. (2001) indicated that RSS are produced by the interaction of ROS with cellular thiol compounds, such as GSH. The specific RSS produced are mono- or disulfide-S-oxides. These species have an oxidation state greater than

ROS (+1 and +3 for mono- and disulfide-S-oxidase, respectively) and require a greater number of electrons to satisfy their reduction (4 or 6 for mono- and disulfide-S-oxidase, respectively), when compared to ROS that seek one or two electrons for their own stabilization.

Under normal cellular conditions, the intracellular and mitochondrial antioxidants, superoxide dismutase (**SOD**; Mn and Cu-Zn), GSH, glutathione peroxidase (**GSH-Px**), catalase, and vitamins C and E, are capable of quenching the ROS formed. However, because the removal of S from the body involves conjugation with GSH this may contribute to a depletion of GSH content in cells. The decrease in GSH content of the cell may increase pressure on the other antioxidants (SOD, GSH-Px, catalase, and vitamins C and E) to maintain homeostasis within the cell and mitochondria. However, because S antagonizes several trace minerals that comprise cellular antioxidants, consumption of a high S diet may deplete the body's stores of antioxidants thus promoting an oxidative environment and cell cytotoxicity.

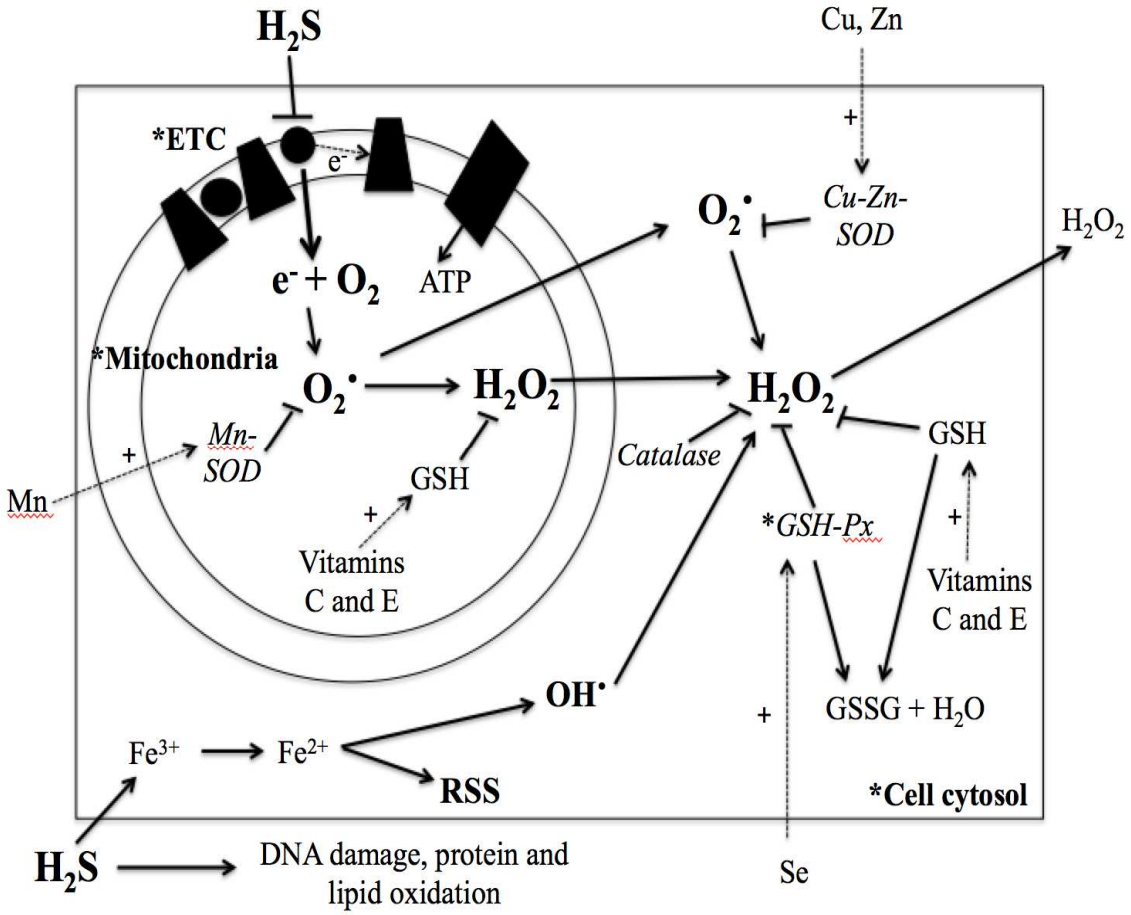


Figure 3. The proposed mechanism by which hydrogen sulfide (H_2S) contributes to cytotoxicity and cell death (Truong et al., 2006), and how trace minerals and vitamins may contribute to alleviating the negative effects imposed by H_2S. Hydrogen sulfide can inhibit cytochrome *c* oxidase of the electron transport chain (ETC), contributing to the production of superoxide ions ($O_2^{\bullet}$) by combination of oxygen with leaked electrons (e^-) of the ETC. The activity of manganese superoxide dismutase (Mn-SOD) and copper-zinc superoxide dismutase (Cu-Zn SOD) converts the superoxide ions to a less damaging compound, hydrogen peroxide (H_2O_2). Hydrogen peroxide is converted to water via glutathione (GSH), selenium dependent glutathione peroxidase (GSH-Px), catalase, and vitamins C and E. Hydrogen sulfide can also contribute to the release of cellular iron (Fe^{2+} and Fe^{3+}) from the iron storage protein ferritin, contributing to reactive sulfur species (RSS) and hydroxyl radical ($OH^{\bullet}$ formation via the Fenton reaction.

Antioxidants in Biological Systems

The antioxidant systems of the body are employed to control the oxidative environment of cells and their surrounding space (extracellular matrix, **ECM**). Combating oxidative damage may be accomplished through antioxidants postponing or averting oxidation or removing damaged tissue (Lykkesfeldt and Svendsen, 2007). Antioxidants are capable of neutralizing radicals through electron donation without becoming dangerous radicals themselves (Rose and Bode, 1993). Neutralization often occurs primarily by interrupting the initiation or progression of oxidation. Specific antioxidants in the body include VC, vitamin E (**VE**), GSH, and the enzymes SOD (Mn and Cu-Zn), catalase, and GSH-Px. The coordinated events of removing radicals is highly dependent on the transfer of electrons to radicals and the availability of reducing agents (NADPH and NADH), often derived from the pentose phosphate pathway (Noctor and Foyer, 1998), to regenerate antioxidants.

Enzymes

As aforementioned, trace minerals contribute to the maintenance of the body's antioxidant capacity through incorporation into the enzymes SOD, catalase, and GSH-Px. Copper, Mn, and Zn contribute to the SOD enzyme, specifically located within the mitochondria (Mn) and cytosol (Cu-Zn). The SOD enzyme catalyzes the dismutation of the superoxide radicals to yield oxygen and hydrogen peroxide (McCord and Fridovich, 1968). The accumulation of hydrogen peroxide, often the result of dysfunctional or depleted antioxidants, can result in tissue damage by spontaneous conversion of hydrogen peroxide to the hydroxyl radical (Arthur, 2000). The body has other systems in place to metabolize

hydrogen peroxide to water, specifically catalyzed by the Fe-dependent catalase and Se-dependent GSH-Px (Arthur, 2000). While antioxidants in the body are often redundant, a deficiency of trace minerals may decrease the antioxidant capacity of the body.

The antagonisms between S and trace minerals may limit the availability of trace minerals for use in antioxidant enzymes, specifically Cu and Se (SOD and GSH-Px). Holstein steer calves (n = 8; 70 kg BW) were used in an 84 d Se depletion study (Se adequate: 0.2 mg/kg diet and Se deficient: 0.03 mg/kg diet in a starter diet) to determine the impact of Se status on immune response to infectious bovine rhinotracheitis virus (Reffett et al. 1988). Steers that were depleted of Se had decreased liver GSH-Px activity (15.8 vs. 108.3 mU/g protein, in Se deficient and adequate steers, respectively) on d 84 of depletion. Circulating GSH-Px concentrations remained depressed in the Se deficient steers after intranasal inoculation with infectious bovine rhinotracheitis virus. Alternately, the Se adequate steers had increased GSH-Px concentrations after inoculation, which the authors postulated is due to the necessity of Se by the enzyme for its function.

Similarly, Boyne and Arthur (1981) induced a Se and Cu deficiency in Friesian calves (n=16). Diets were designed to evaluate two concentrations (adequate and deficient) of both Se (added as sodium selenite) and Cu (added as Cu sulfate), for a total of four dietary treatments including: 1) Se and Cu deficient (0.01 mg Se and 1.80 mg Cu/kg diet), 2) Se deficient and Cu adequate (0.01 mg Se and 12.0 mg Cu/kg diet), 3) Se adequate and Cu deficient (0.1 mg Se and 1.80 mg Cu/kg diet), and 4) Se and Cu adequate (0.1 mg Se and 12.0 mg Cu/kg diet) for a period of 25 weeks, after which steers were kept on pasture for the remaining three weeks of the trial (pasture contained 0.001 mg Se and 7.05 mg Cu /kg DM). After nine weeks of consuming a Se-deficient diet, the neutrophil GSH-Px activity of the Se-

depleted calves decreased to undetectable limits for the remaining 19 weeks on study in comparison to the enzyme activity of the Se supplemented group which maintained neutrophil GSH-Px activity between 5 to 8 $mU/10^6$ cells throughout the study entirety. This study also examined the impact of a concurrent Cu deficiency to the Se deficiency, and noted no impact of Cu deficiency on neutrophil enzyme activity (GSH-Px or SOD), suggesting Cu-Zn SOD activity of neutrophils might not be very sensitive to changes in Cu status (Arthur and Boyne, 1985).

These authors reported neutrophil function of these steers at a different time (Boyne and Arthur, 1981). The capacity of neutrophils from Se-depleted steers to engulf and kill bacteria (specifically *Candidia albicans*) decreased sharply within the first nine weeks of the study, similar to the decline of GSH-Px, and by week 25 the Se adequate-Cu deficient steers began to show a similar decline in neutrophil killing capacity; however, the Se and Cu adequate steers maintained killing capacity throughout the 28 week study. Alternately, Xin et al. (1991) reported that a depletion of liver Cu status of Holstein steers (n = 12; 211 kg BW), by Mo antagonism (10 mg Mo/kg diet DM) for 8 mo, decreased the Cu concentration of neutrophils and decreased SOD activity in whole blood, red blood cells, and neutrophils. Similar to Boyne and Arthur (1981), Xin et al. (1991) noted a decreased Cu status decreased the killing capacity of neutrophils (27.7 and 17.3% for Cu adequate and Cu deficient steers, respectively), which authors attribute to a decreased SOD activity in the neutrophils.

Vitamins and glutathione

The antioxidant roles of VC and VE have been well documented, as the structures of these vitamins make them suitable for protection of both lipid membranes (VE) and the

aqueous environment of the cell and ECM (VC). The auto-oxidation reaction, by which ROS seek to stabilize their own structure, occurs by three steps: initiation, propagation, and termination. Cell membranes, especially those containing unsaturated fatty acids, are highly susceptible to oxidation by ROS due to the weak double bond interactions of the carbon and hydrogen atoms. This weakened interaction enables ROS to extract electrons to stabilize their own structure, resulting in the production of a lipid-derived radical, which can initiate auto-oxidation. The propagation step occurs as the newly formed lipid radical seeks to stabilize itself by removing electrons from neighboring fatty acids. The lipid soluble nature of VE enables its incorporation into the lipid bilayer, approximately 40% surrounds nuclear membranes and 60% is incorporated around other lipid membranes of the cell (Traber and Atkinson, 2007). Vitamin E is able to terminate the propagation step of auto-oxidation by donating electrons to stabilize the lipid radical; however, in the process of removing radicals VE is sacrificed (becoming a radical itself, tocopheroxyl-radical) and must be removed from the membrane or be regenerated to its reduced form (α-tocopherol; Traber and Stevens, 2011).

The water-soluble nature of VC allows mobility within the cell and surrounding environment, thus enabling it to regenerate other antioxidants, such as VE and GSH. In addition to the ability to rescue other antioxidants, VC is able to neutralize myriad of radical species, including the hydroxyl radical. The reducing action of VC occurs through its ability to donate one or two electrons, forming the intermediate radical semi-dehydroascorbic acid or the oxidized form of ascorbic acid, dehydroascorbic acid (**DHA**), respectfully. The regeneration of the reduced form of VC is dependent on the surrounding antioxidants and

reductants (GSH, NADPH, and NADH) and the enzymes semi-dehydroascorbic acid reductase and DHA reductase.

The reducing capabilities of GSH are attributed to the ability to form disulfide bonds upon oxidation, via the S-containing cysteine residues. From each GSH molecule one electron is available for donation to ROS; therefore, two molecules are required in reducing reactions to yield glutathione disulfide (**GSSG**, or "oxidized GSH"). Glutathione disulfide can be regenerated to GSH through the enzymatic activity of GSH-reductase, requiring FAD^+ as a cofactor and reducing equivalents from NADPH. In normal, healthy tissue GSSG should comprise no more than approximately 10% of the total GSH, as percentages exceeding 10% are indicative of oxidative stress (Ithayaraja, 2011). In regard to S-induced oxidative stress, Truong et al. (2006) exposed cultured rat hepatocytes to sodium hydrosulfide (to simulate H_2S) and observed a depletion of GSH, suggesting that GSH is essential to the detoxification process of excess S. The depletion of GSH by conjugation with S may limit the availability of GSH to participate in other critical antioxidant functions in the body and increase the strain on other body antioxidants such as vitamins C and E, SOD, catalase, and GSH-Px.

Oxidative Stress and Live Animal Performance and Meat Quality

An excessive oxidative environment can hinder feedlot performance and the carcass value of cattle, translating to a loss in profit for producers (Duff and Galyean, 2007). The development of an oxidative environment in feedlot cattle can arise from several factors including: weaning, transportation, environment, co-mingling, and handling practices at the feedlot. Chirase et al. (2004) reported that the transportation of beef calves over 3,500 km to the feedlot resulted in decreased serum total antioxidant capacity and increased serum

malondialdehyde (**MDA**), and stressed cattle were at a greater risk of incidences of bovine respiratory disease and mortality. Stressors often result in lesser DMI and therefore a lesser consumption of necessary nutrients, such as trace minerals and vitamins, for sustained health. As a means to combat the decreased DMI of newly received cattle, the NRC (2000) suggests increasing the concentration of mineral in the receiving diet 1.5 times the requirement to increase the likelihood of cattle meeting their mineral needs even with a reduced DMI. Supplementation of VE at 1,140 IU/d (Rivera et al., 2002; n = 200 beef heifers, 204 kg BW) or 2,000 IU/d (Carter et al., 2002; n = 387 beef heifers, 197 kg BW) for 28 d decreased medical costs (primarily decreasing the cases of bovine respiratory disease), but did not influence live performance of cattle, DMI, ADG, or feed efficiency (Rivera et al., 2002; Carter et al., 2002).

An oxidative environment in postmortem muscle can be residual from live animal conditions or can arise from the exposure to irradiation, presence of oxygen in the packaging, or endogenous components of muscle, such as heme-Fe, unsaturated fatty acids, or transition metals (Rowe et al., 2004a,b; Kim et al., 2010; Estevez et al., 2011). An alteration of protein structure by oxidation can have repercussions on its functionality through aggregation, fragmentation, loss of solubility, and/or enzyme activity (Xiong and Decker, 1995). Protein oxidation can decrease muscle tenderness specifically by altering protein structure and function (aggregation, fragmentation, and crosslinking) and loss of enzyme activity and specificity to substrates (Stadman and Levine, 2000; Estevez, 2011). Protein carbonyl formation has been acknowledged as an indicator of protein oxidation during times of oxidative stress, aging, or disease states (Stadman and Levine, 2000). The posttranslational modification of carbonyl formation results from the interaction of muscle proteins with ROS,

especially in the presence of transition metals (Cu and Fe), by the irreversible oxidation of the protein side-chains containing arginine, lysine, proline, threonine, cysteine, and methionine. The direct oxidation of proteins involves the ROS extraction of hydrogen from the amino group's neighboring carbon, resulting in the formation of a carbon-centered radical. These radicals can react with other proteins in the presence of oxygen, transition metals (Cu or Fe), or other ROS to propagate autoxidation. Oxidized transition metals can interact with hydrogen peroxide to produce more reactive radicals, such as the hydroxyl radical, via the Fenton reaction (Fe^{2+} + hydrogen peroxide yields hydroxyl radical) and the precursor reaction, the Haber-Weiss reaction (Fe^{3+} + superoxide radical yields Fe^{2+}). Since the formation of the carbonyl group is irreversible, oxidized proteins are often marked for degradation by cellular proteases and ubiquitin (Stadman and Levine, 2000).

The consequence of carbonyl formation, in regards to meat quality, is primarily associated with the loss in functionality of oxidized proteins and greater amounts of crosslinking. Together these factors may alter the ability of the proteins to be targeted for degradation by muscle proteases, thereby limiting overall protein degradation and increasing toughness of steaks. A positive correlation between protein carbonyl content and Warner-Bratzler shear force values of irradiated beef steaks has been reported by Rowe et al. (2004a). While the exact mechanism by which protein carbonyls affect meat quality remains unclear, Estevez (2011) suggested that protein oxidation may be hindering the tenderization process by decreasing proteolytic degradation or by the formation of disulfide bonds. Oxidation of both muscle proteins and enzymes can decrease the ability of the enzyme to recognize their substrates or induce their inactivation, especially if the active site of the enzyme is composed of S-containing amino acids (cysteine and methionine). Additionally, accumulation of

crosslinks by oxidized proteins may result in a strengthening of the muscle (decreasing tenderness) or may result in myofibrillar shrinkage (decreasing water holding capacity and juiciness).

Postmortem tenderization of muscle is highly dependent on the activity of proteases to degrade proteins within the muscle fiber. The calpain proteases are Ca-dependent enzymes that participate in the degradation of skeletal muscle, in both ante-mortem (tissue turnover) and postmortem (tenderization) situations (Koohmaraie, 1992; Goll et al., 2003; Stolowski et al., 2006; Veiseth-Kent et al., 2010). The names of the calpains, 1 (formerly µ-calpain) and 2 (formerly m-calpain), are in reference to their Ca requirement for half-maximal activity; calpain-1 ranges from 3 to 50 µM Ca and calpain-2 ranges from 300 to 1000 µM Ca (Goll et al., 2003). Calpain undergoes autolysis, or self-degradation, in which the mass of the catalytic subunit of the enzyme is sequentially reduced from 80-kDa (indicating an intact protein) to 78-kDa (an intermediate product), and finally to the 76-kDa (fully autolyzed) subunit. Undergoing autolysis decreases the Ca required for half-maximal activity (0.5 to 2.0 and 50 to 100 µM, calpain-1 and -2, respectively); however, the exact role of autolysis in regards to enzyme activation is still not clear (Baki et al., 1996; Hongqi et al., 2004). The autolyzed form of the enzyme is most often and consistently observed during proteolytic activity by calpains. Thus, calpain autolysis is utilized as a marker of the history of calpain's proteolytic activity (Baki et al., 1996; Goll et al., 2003).

Calpains degrade a vast variety of proteins associated with muscle fibers, including titin, desmin, vinculin, but specifically target tropomyosin and the troponin complex [T (tropomyosin associated), I (inhibitory), and C (calcium binding)], which are proteins closely associated with actin filaments. However, there are several factors within muscle that can

influence the activity of calpain, including: calcium availability, the rate and extent of pH decline, an oxidative environment, and the activity of calpastatin, the inhibitor of calpain (Goll et al., 2003; Lametsch et al., 2008).

The active site of calpain-1 contains cysteine residues, which are highly susceptible to oxidation, and the formation of a disulfide bond between the cysteine residues 108 and 115 (Lametsch et al., 2008). Several authors have reported the presence of an oxidizing environment (primarily hydrogen peroxide) interferes with calpain's ability to exert proteolytic and autolytic activity (Guttman and Johnson, 1998; Rowe et al., 2004; Lametsch et al., 2008). Lametsch et al. (2008) determined the activity of calpain-1 after incubation with 200 µM hydrogen peroxide and either 0.03 or 0.1% merceptoethanol, a reducing agent used to prevent formation of disulfide bonds. These authors noted observed calpain activity and autolysis decreased after exposure to hydrogen peroxide when compared to calpain incubated with both hydrogen peroxide and merceptoethanol. Under reducing conditions, the pre-exposure of calpain to an oxidizing environment had no impact on the activity of calpain, suggesting the disulfide bond formed between the cysteine resides is reversible (Lametsch et al., 2008).

Troponin T is a component of the troponin complex which is involved in the formation of actin-myosin cross bridges during contraction. The actin-myosin cross bridge formation is facilitated by the conformational change induced by the binding of calcium to troponin C, which enables troponin T to bind and move tropomyosin away from the myosin active site on actin. The troponin complex is especially susceptible to protein degradation by the calpain system, making it an excellent marker for protein degradation within the muscle (Huff-Lonergan et al., 2010). The presence of an oxidative environment can hinder

proteolytic activity and the subsequent tenderization of meat, as a positive relationship has been identified between the extent of troponin T degradation, myofibrillar fragmentation index, and shear force values (Olson and Parrish, 1977; Huff-Lonergan et al., 1996).

The addition of exogenous antioxidants to combat the development of oxidative conditions in the postmortem muscle has been highly investigated (Nam and Ahn, 2003; Realini et al., 2004; Rowe et al., 2004a,b). However, researchers have primarily been concerned with the lipid soluble VE, as this vitamin has a greater storage capacity and half-life within the body when compared to the water soluble VC. Rowe et al. (2004a) supplemented 20 beef steers (396 kg BW) with 1000 IU VE·steer^{-1}·d^{-1} for the entire 126 d finishing period to determine the ability of VE to modulate the development of an oxidative environment after exposure of beef strip loin steaks to irradiation (inducing an oxidative environment). At 2 days post-irradiation, increases in troponin T degradation and decreases in protein oxidation were observed in the irradiated steaks from cattle supplemented with VE. These results suggest the supplementation of a lipid-soluble antioxidant may be beneficial to combat protein oxidation (of muscle fibers and enzymes) in postmortem muscle, to allow the tenderization process to occur.

The pigmentation of meat is owed to the oxidation state of the myoglobin heme-Fe and the ligand bound to the side chain of the porphyrin ring (Brewer, 2004). In fresh meat, chemical forms of myoglobin that may be detected include: the cherry-red pigment oxymyoglobin (O_2 bound), purple pigment deoxymyoglobin (nothing bound), the brown-pigment metmyoglobin (H_2O bound), and very-bright cherry red pigment of carboxymyoglobin (CO bound). Two others, sulfmyoglobin (H_2S bound) and cholemyoglobin (H_2O_2 bound) are capable of forming, both resulting in a green

pigmentation. Deoxymyoglobin predominates in anaerobic situations, such as intact muscle or vacuum packaged meat, but the presence of oxygen triggers a “blooming” effect to yield oxymyoglobin. The discoloration of meat, or the formation of metmyoglobin, results when the oxidation state of the Fe changes from ferrous (Fe^{2+}) to ferric (Fe^{3+}) and water binds the free ligand position (Brewer, 2004). The lifespan of oxymyoglobin (maintaining Fe^{2+}) is dependent on the enzyme metmyoglobin reductase and the availability of reducing equivalents, such as NADH (Moller and Skibsted, 2006).

During the conversion of muscle to meat, the continuation of glycolytic processes decreases muscle pH. As muscle pH nears the iso-electric point of muscle the attraction of water to muscle proteins is decreased, and thus increasing “free” water or purge and increasing water on the surface of meat (Huff-Lonergan and Lonergan, 2005). The reflectance of water on the surface of meat can influence the coloration perceived by consumers, as myoglobin is a water-soluble protein. Additionally, because a higher ultimate pH allows the retention of water by muscle proteins the fibers are more tightly packed and limit/slow the interaction of oxygen with myoglobin, thus meat has a darker appearance (Renerre and Labas, 1987).

Measures of Oxidative Stress

The evaluation of oxidative stress can be categorized by the presence of oxidants/antioxidants or the assessment of oxidative damage (DNA, protein, lipid, etc.; Lykkesfeldt and Svendsen, 2007). Evaluation of the antioxidants GSH and ascorbate in blood or tissues may be a useful marker to determine antioxidant status of an animal, as these molecules are involved in the maintenance and regeneration of other lipid-soluble

antioxidants, such as VE, and may be evaluated by high-performance liquid chromatography (Haiying et al., 2003; Padilla et al, 2007; Matsui, 2012) or the determination of the ratio of oxidized-to-reduced GSH (Ithayraja, 2011). Ascorbate may also be assayed in plasma by conversion of ascorbate to the oxidized DHA, and then condensed with a fluorescent product for analysis via fluorescent spectrometry (Cayman Chemical Company). However, these compounds are highly susceptible to oxidation and may oxidize prior to analysis, making their evaluation potentially difficult. Decreases in the presence of these two antioxidants may be useful as indicators of oxidative stress.

The measurement of total antioxidant capacity indicates the measure of all antioxidant compounds within the blood or tissue, including enzymes, small proteins (albumin, ceruloplasmin, and ferritin), and small molecules (VC, VE, GSH, uric acid, and bilirubin). Specifically within blood, these will be endogenous and dietary derived antioxidants. The measure of total antioxidant capacity is a non-specific, colormetric measure of antioxidants within circulation, including vitamins C and E, carotenoids, flavonoids, and phenolic compounds and their ability to prevent oxidation of a specific compound (such as hemoglobin) to its oxidized form (methemoglobin; Prior, 2004). Alternately, Abuelo et al. (2013) were the first to identify the oxidative stress ratio in serum of dairy cattle (d-ROM test and OXY-Adsorbent Test, Diacron International). These assay determine a ratio of pro-oxidants and oxidants in circulation, which is a more sensitive measure than the use of either pro-oxidants or antioxidant alone. These two assays spectrophotometrically determine the hydroperoxides resulting from the breakdown of lipids and other organic material caused by ROS attack and the cumulative action of all antioxidants in the serum.

Within tissues, the measurement of oxidative stress is often evaluated by detection of byproducts of oxidation, such as DNA damage or protein and lipid oxidation. A common method to evaluate lipid oxidation is via thiobarbituric acid reactive substances (**TBARS**; Valenzuela, 1991). This assay determines the MDA produced during the oxidative degradation of polyunsaturated fatty acids (**PUFA**). The MDA product can be determined from many biological samples, such as tissue homogenates, blood (plasma/serum), or urine. Specifically, the assay involves the derivitization of one MDA with two thiobarbituric acid molecules, which can then be measured colormetrically as the MDA-TBA adduct forms a pink pigment (Sinnhuber and Yu, 1958).

The evaluation of protein carbonyls, which occur by the direct oxidation of amino acids by ROS, is the most common method to determine oxidative damage (Stadman and Levine, 2000; Lykkesfeldt and Svendsen, 2007). Protein carbonyl formation can arise from oxidative cleavage of the protein backbone or the interaction of muscle protein with ROS resulting in a posttranslational modification of muscle amino acids specifically arginine, lysine, proline, threonine, cysteine, and methionine (Stadtman, 1992). However, it is important to note that the presence of carbonyl groups in protein is not a sole indicator of oxidative modification, as the addition of sugar molecules to amino acids (glycosylation) or interaction with the aldehyde byproducts of lipid oxidation (MDA) may introduce carbonyl groups (Levine et al., 2000). Assays to determine protein carbonyl content may be via colorimetric or immunodetection methods. However, both methods involve the derivitization of proteins with 2, 4-dinitrophenylhydrazine (DNPH) to forming a protein-DNP hydrazone moiety, which provides a strong absorbance when it interacts with carbonyl groups (Castegna

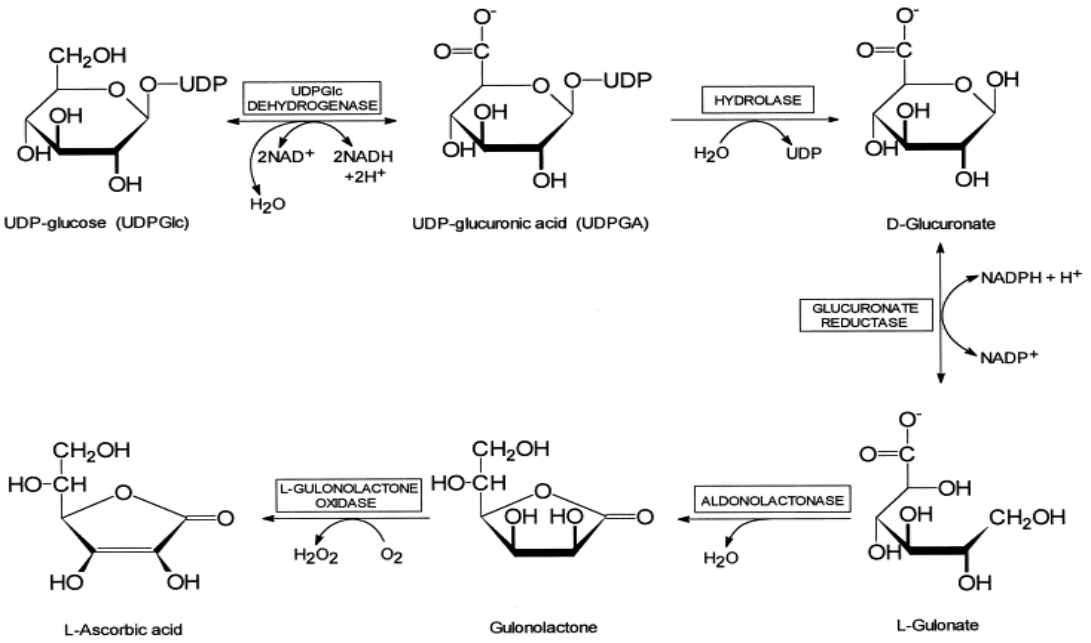
et al., 2003). Alternately, antibodies specific for the DNP moiety of the proteins can be utilized to determine the carbonyl content (OxyBlot Kit, Millipore).

Summary of Oxidative Stress

It is well established that an oxidative environment has negative ramifications in both live and postmortem situations. While the role of S in production and health has been extensively examined, live animal research data concerning the contribution of S to the development of oxidative stress and the residual effects on meat quality are lacking. Recent cell culture work does support the idea that H_2S contributes to oxidative stress, via the depletion of cellular GSH (Truong et al., 2006). Given the interrelationship between GSH and VC and the redundancy that exists among antioxidants in the body, the depletion of GSH by S may increase the body's demand for VC.

Vitamin C

Vitamin C has many roles in cellular metabolism, but it specifically aids in oxidation-reduction reactions and acts as an enzyme cofactor for the synthesis of collagen, L-carnitine, and norepinephrine (Rebouche, 1991; Combs, 2008). The NRC (1996) does not specify a requirement for the supplementation of VC in cattle diets, as cattle can synthesize ascorbic acid (vitamin C) from glucose in the liver via the hexuronic acid pathway, illustrated in Figure 4 (Banhegyi et al., 1997). This process is made possible by the final enzyme, L-gulonolactone oxidase, in the conversion of glucose to ascorbic acid. Through evolutionary loss, L-gulonolactone oxidase is not present in humans, primates, bats, and some species of birds and fish, indicating these species require exogenous VC to meet dietary needs.



Banhegyi et al., 1997

Figure 4. The synthesis of ascorbic acid from glucose in mammalian species.

Tsao and Young (1990) suggest endogenous VC production by species without an exogenous VC requirement may be decreased with dietary VC supplementation. These authors supplemented 0.5, 1, or 5% ascorbic acid to the diet of mice, and measured the concentration of VC entering the portal blood. Exogenous ascorbic acid intake decreased the production of VC in the liver of rats. The liver concentration of ascorbic acid and the rate of synthesis were not statistically correlated, which suggests that VC production was not altered by liver concentration of the vitamin. However, a strong negative correlation was detected between the portal blood concentration of ascorbic acid and the rate of VC synthesis in the liver of rats, indicating the synthesis of VC in the liver is dictated by VC concentration of the portal blood or dietary sources. Similar data are not available in ruminant species; however, if a similar response were to occur, this would indicate supplemental VC could act as a

sparing mechanism for glucose to be potentially directed toward growth and lipid accumulation, rather than production of the vitamin.

Vitamin C Bioavailability and Transport

Vitamin C is primarily found as ascorbic acid (80 to 90%) in feeds, but may also be present in the oxidized form, DHA (Combs, 2008). Many plants have the ability to synthesize ascorbic acid from carbohydrates, and some species have a greater capacity to store the vitamin such as fresh tea leaves, berries, guava, and rosehips. The stability of VC may be compromised by various processing methods, specifically those involving the application of heat or the general deleterious effects of time. Exposure to oxidative conditions irreversibly convert ascorbic acid to 2,3-diketogulonic acid, the biologically inactive form.

Because VC is highly susceptible to oxidation by external sources (environment, heat, etc.), specifically in aqueous conditions with neutral pH at 37°C (Takamizawa et al., 2004), attempts to increase the stability of VC for use in feed and culture media have been examined. The addition of a phosphate group to the 2-hydroxyl group, forming L-ascorbyl-2-phosphate or L-ascorbic acid 2-phoshate, protects L-ascorbic acid from being oxidized (Grant et al., 1989; Takamizawa et al., 2004). Grant et al. (1989) reported L-ascorbyl-2-polyphosphate lost approximately 15-22% activity after the process of milling and storing, and the stability following 25 and 40°C pelleting was 83 and 46 times, respectively, greater than L-ascorbic acid. Additionally, during steam-pelleting and shipping on dry ice, L-ascorbyl-2-polyphosphate was not changed, while a 46% loss in L-ascorbic acid was noted (Grant et al., 1989). Takamizawa et al. (2004) reported ascorbic acid 2-phosphate was a long

acting stable ascorbic acid derivative for use in cell culture. Alkaline phosphatase activity of the cells liberates ascorbic acid for absorption into cells.

Within the rumen, Knight et al. (1941) reported the complete destruction of ascorbic acid 2 hours post-ingestion, and using *in vitro* models, ascorbic acid was destroyed within 4 hours. Zinn et al. (1987) used three crossbred steers (194 kg BW), within rumen and proximal duodenal cannulae, in a 3 × 3 Latin Square design to determine the impact of ascorbic acid supplementation (0, 100, or 1,000 mg ascorbic acid·steer^{-1}·d^{-1}) on digestion and microbial efficiency. In this study, no ascorbic acid was detected in the duodenum, indicating that no orally consumed ascorbic acid reached the portal blood stream of ruminant species. Therefore, the use of a rumen-bypass source or intravenous infusion of VC is required to reach the portal blood stream of ruminants.

Encapsulation of VC is one method to limit the degradation of VC in the rumen, and one such product commercially manufactured is Vitasure C by Balchem Corporation (New Hampton, NY). This VC product contains 50% ascorbic acid coated with a lipid coating, consisting of hydrogenated vegetable oil. Garrett et al. (2007) evaluated the stability of encapsulated VC (Balchem Corporation) compared to raw ascorbic acid via *in vitro* rumen fermentation. Over the 24-hour incubation period, the two encapsulated VC sources maintained approximately 50% viability, whereas the raw ascorbic acid product was almost entirely destroyed within 6 hours.

While no information is available concerning the requirement of VC by rumen microorganisms, a recent short communication by Tagliapietra et al. (2013) indicated the addition of 8 mg L-ascorbic acid/g of feed sample to an *in vitro* rumen fermentation of corn increased volatile fatty acid production, specifically acetate (1.33 and 1.55 mmol/g DM, from

the control and VC included, respectively) and propionate (0.67 and 0.78 mmol/g DM, from the control and VC included, respectively), and gas production (143 and 174 ml/g DM, control and VC included, respectively) compared to the control. The authors suggested that VC might be promoting a greater or faster degradation of substrates compared to the control, specifically toward the production of gas and volatile fatty acids rather than microbial mass.

Because VC is a large, polar molecule it requires assistance to travel through membranes (Li and Schellhorn, 2007). Within the body several transport mechanisms for VC are present and have been well documented, including sodium-dependent VC transporters (**SVCT**) 1 and 2, the hexose (glucose) transporters (**GLUT**), and passive diffusion across cell membranes. The absorption of ascorbate is often Na-dependent, while DHA absorption is a Na-independent cell-trapping method (Figure 5; Rumsey and Levine, 1998). Within the body, VC is found in the greatest concentrations within the adrenal and pituitary glands, followed by the liver, thymus, brain, and pancreas (Rucker et al., 2008).

In species with the ability to synthesize VC from glucose in the liver, which includes cattle, it has been presumed that consumed VC will be transported across the small intestine via passive diffusion (Combs, 2008). However, mRNA for the SVCT system has been identified in the small intestine of mice, a species also capable of synthesizing VC (Amano et al., 2010). This information may indicate the presence of SVCT transporters in the small intestine of cattle; but this has not been determined conclusively. While the whole genome of the domestic cow (*Bos taurus)* has been sequenced and genes for both SLC23A1 and A2 have been identified, no differential expression or tissue specific expression has been confirmed. However, these genes have been cloned and high conservation of functional

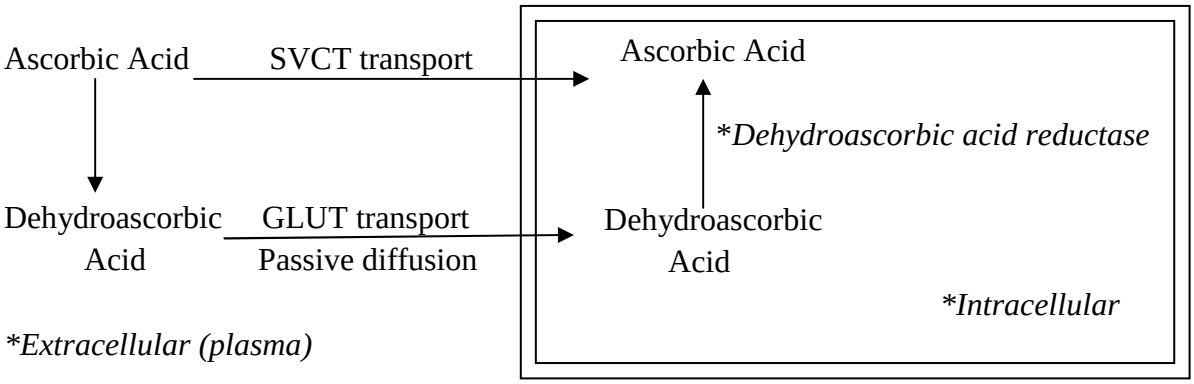
expression of the gene has been noted among humans, mice, rats, dogs, and swine (Michels, 2007).

Sodium dependent-VC transporters are the primary mode of cellular uptake of ascorbate, as these transporters have the highest affinity for ascorbate. The SVCT are a 12-transmembrane complex requiring a Na electrochemical gradient to transport VC into the cell (Wang et al., 2000; Wilson, 2005), specifically exchanging two Na^+ molecules for one ascorbate molecule (Wilson, 2005) and maintaining Na concentrations via Na-K ATP*ase* along the basolateral membrane. This transporter exists in two isoforms, SVCT-1 and SVCT-2. Sodium-VC transporter 1 is primarily involved in maintaining whole body VC homeostasis, evident by its localization in the endothelial cells of the intestine, liver, and kidney; whereas, SVCT-2 has been identified in specialized cells, such as the brain, eye, and placenta (Tsukaguchi et al., 1999; Savini et al., 2005; Wilson, 2005). Interestingly, although 40% of the body's VC is stored in muscle, no SVCT-1 and very little SVCT-2 mRNA has been detected in muscle cells (Wang et al., 2000; Kuo et al., 2004), but rather, the importation of VC into skeletal muscle cells may be reliant on the GLUT transport system and passive diffusion (Wang et al., 2000). Kuo et al. (2004) concluded the SVCT isoforms act independently of one another to maintain tissue ascorbate concentrations. Both forms of SVCT are regulated by concentrations of VC within the cell. As the up-regulation of SVCT-1 and GLUT-4 in the small intestine have been observed in VC inadequate diets (Amano et al., 2010), while the presentation of large quantities of VC can inhibit and/or down regulate the presence of SVCT on the cell surface (Combs, 2008).

Transportation of DHA, the oxidized form of VC, can be facilitated by two means: passive diffusion (driven by a concentration gradient) or by the GLUT family of transporters

(1, 3, and 4). When ascorbic acid is oxidized (DHA) it is immediately taken up by cells and reduced to ascorbic acid. This process creates a concentration gradient to ensure the maintenance of reduced VC in the extracellular environment (Rumsey and Levine, 2008). Transportation by the GLUT system is driven by a gradient which requires a three step process (Li and Schellhorn, 2007), specifically outlined as: 1) oxidation of extracellular ascorbate (conversion to DHA), 2) transportation of DHA across the cell membrane through various GLUT transporters: GLUT1 (brain, mitochondria), GLUT3 (nerve cells), or GLUT4 (adipocytes), and 3) reduction of DHA back to ascorbate within the cell via DHA reductase.

As glucose and DHA share a transporter, during times of increased availability of glucose DHA transport by GLUT is competitively inhibited. Savini et al. (2005) suggested that because of this competition, GLUT transporters are unlikely to play a role in VC uptake into cells, as the transport of glucose is a greater priority. However, these authors also suggested that during times of oxidative stress this transport mechanism might become necessary to maintain VC concentrations in the cells. In addition to glucose competition, GLUT transporters are under hormonal regulation, specifically follicle stimulating hormone, insulin-like growth factor 1, and insulin (Kodaman and Behrman, 1999). Li and Schellhorn (2007) suggest measurement of both serum glucose and ascorbic acid concentrations may be beneficial in understanding availability of VC in cells.



Adapted from Combs, 2008

Figure 5. Absorption and recycling of ascorbic acid witin the cell. Ascorbic acid may be transported into target cells by the sodium dependent transports (SVCT-1 and SVCT-2) for utilization within the cell, or upon oxidation of extracellular ascorbic acid to dehydroascorbic acid (DHA) the glucose transporters (GLUT) or passive diffusion can facilitate the movement of DHA into the cell for reduction to ascorbic acid by DHA reductase.

Vitamin C in Circulation

Ascorbic acid is the dominant form of VC found in circulation; by contrast, DHA is either non-detectable or not found in circulation because of the rapid uptake by cells for reduction to ascorbic acid (Rumsey and Levine, 1998). Limited information is available concerning the normal concentration of circulating VC in feedlot cattle; however, available research data indicate a wide range in plasma VC concentrations among cattle and across all aspects of production. A review by Smith et al. (2009) summarized the plasma VC concentrations of healthy beef cattle to range from 2,400 to 4,700 µg/L (13.6 to 26.7µmol/L). Knight et al. (1941) reported plasma VC concentrations of 2,800 to 5,900 µg/L (15.9 to 33.5 µmol/L) from three Holstein cows injected intravenously with 24 g ascorbic acid (in water), and Hidiroglou et al. (1977) reported average plasma VC concentrations of 1,100 to 1,400 µg/L (6.2 to 7.9 µmol/L) from 60 yearling non-VC supplemented Hereford steers during the

winter months of finishing. Additionally, Hidiroglou et al. (1977) observed that the cattle housed indoors and consuming a high grain diet during the winter months, displayed the greatest peak in plasma VC concentrations between the months of November and December (1,700 to 2,200 µg/L for hay and grain treatments, respectively) compared to those maintained on a forage based diet (1,300 to 1,400 µg/L for grain and hay treatments, respectively). Both of these studies (Knight et al., 1941 and Hidiroglou et al., 1977) assayed plasma VC via the photoelectric colorimeter assay as described by Mindlin and Butler (1938). This method consists of a colormetric assessment plasma ascorbate concentrations by reduction reaction between ascorbate and a 2,6-dichlorophenol indophenol (an oxidizing dye) after a 30 second reaction time. The concentrations of plasma ascorbate are determined by subtracting a blank sample value from the sample value, and multiplying this number by a calculated constant (measuring varying concentrations of crystalline ascorbic acid dissolved in 2.5% metaphosphoric acid). The final values are the mg of ascorbic acid per 100 mL of plasma.

The use of high-performance liquid chromatography has been employed to determine the VC concentration of bovine plasma (Haiying et al., 2003; Padilla et al, 2007). Haiying et al. (2003) observed plasma VC concentrations to range from 1,500 to 3,300 µg/L (8.5 to 18.9 µmol/L) in 10 fattening steers (not supplemented with VC), while Padilla et al. (2007) reported plasma VC concentrations ranging from 2,670 to 4,080 µg/L (15.2 to 23.2 µmol/L) in five, 3 to 4 year old cows supplemented with a rumen-protected VC source.

In finishing cattle, Takahashi et al. (1999) reported a wide range of final serum VC concentrations (810 to 16,784 µg/L; 4.6 to 95.3 µmol/L) in 78 Japanese-Black cattle collected at the slaughterhouse. This variation inspired a study to investigate the VC

concentration throughout the finishing period. Four Japanese long-fed cattle (not supplemented with VC) were used to track circulating VC concenrations throughout the finishing period (approximately 19 months). The authors reported a decrease in serum VC concentrations from approximately 15,000 to 4,600 µg/L (85 to 26 µmol/L) across the first 12 months of the finishing period, while VC concentrations recovered to approximately 7,900 µg/L (45 µmol/L) through the remainder of the fattening period (7 months) in cattle supplemented with a rumen by-pass VC product.

Padilla et al. (2007) evaluated supplementation of a rumen-protected VC source (coated in hydrogenated soybean oil) at concentrations of 0, 10, 20, 40, or 60 mg VC·kg $BW^{-1}\cdot d^{-1}$ (equivalent to approximately 0, 6, 11, 24, or 36 g of VC·$cow^{-1}\cdot d^{-1}$) to five, 3 to 4 year old Japanese-Black × Holstein cows utilizing a 16 d, 5 × 5 Latin Square design. Vitamin C was supplemented for 9 d in a concentrate-based premix prior to morning feeding. Blood samples were collected on d 7 of VC supplementation, before the VC premix was consumed. Plasma VC concentrations linearly increased in response to increasing concentrations of supplemental VC, and values ranged from 2,670 to 4,080 µg/L (15.2 to 23.2 µmol/L). The authors noted an increase in plasma VC concentrations of approximately 510 to 1,410 µg/L (2.9 to 8.0 µmol/L) with the inclusion of 20, 40, or 60 mg VC·kg $BW^{-1}\cdot d^{-1}$ (11, 24, or 36 g·$cow^{-1}\cdot d^{-1}$) compared to the controls (0 mg VC·kg $BW^{-1}\cdot d^{-1}$), potentially indicating a minimum inclusion of 11 g·$cow^{-1}\cdot d^{-1}$ is necessary to improve VC status of cows. Correspondingly, a dose of 20 mg VC·kg $BW^{-1}\cdot d^{-1}$ or greater linearly increased urinary excretion of VC (0.69, 0.88, and 1.36 mol/mol of creatinine for 20, 40, and 60 mg VC·kg $BW^{-1}\cdot d^{-1}$, respectively) compared to the control and 10 mg VC·kg $BW^{-1}\cdot d^{-1}$ (0.26 and 0.27 mol/mol creatinine, respectively), suggesting a renal threshold, above which circulating VC

is excreted via urine. While the authors were unsure of the exact quantity of the hydrogenated soybean oil coated VC that is being absorbed from the small intestine, authors estimate, based on plasma concentrations and urinary excretion of VC, that approximately 50% of the VC source is available for absorption in the small intestine.

Influence of Vitamin C on Iron and Copper Absorption and Metabolism

It is well accepted that the presence of ascorbic acid in the small intestine enhances the uptake of Fe into enterocytes (Milne and Omaye, 1980; Hallberg et al., 1989). This uptake is facilitated by the reduction of ferric Fe to ferrous Fe by ascorbic acid, as the ferrous state of Fe is recognized and translocated into the enterocytes by divalent metal transporter-1 (DMT-1; Gunshin et al., 1997). Interestingly, within healthy humans, ascorbic acid is concentrated in gastric juice, thus increasing availability for reduction reactions (ROS scavenging and mineral absorption; Rathbone et al., 1989). However, similar information regarding ascorbic acid content of gastric juice in livestock species has not been determined. Milne and Omaye (1980) supplemented guinea pigs, which are unable to synthesize VC, with 0.5 or 25 mg/100 g body weight of vitamin C and a corresponding increase in hepatic and serum Fe and heme, respectively, were noted as VC increased.

While VC enhances the absorption of Fe from the small intestine, the opposite occurs with Cu. Intestinal Cu absorption and metabolism is depressed with ascorbic acid supplementation in chicks, rodents, rabbits, and guinea pigs (van Campen and Gross, 1968; Milne and Omaye, 1980; Disilvestro and Harris, 1981; van den Berg and Beynen, 1992). Copper absorption from the small intestine primarily occurs via the transporter protein Cu-transporter 1 (Lönnerdal, 2008), which facilitates the movement of Cu^{+}, indicating that Cu

must be reduced from the 2^+ state before absorption. However, Arredondo et al. (2003) indicated that DMT-1 may be responsible for approximately 50% of the intestinal transport of Cu into the enterocytes. Contrary to *in vivo* work, Arredondo et al. (2003) indicated that within human cultured Caco-2 cells (human epithelial colorectal adenocarcinoma cells) the presence of ascorbate (500 µM) was required for the apical transport of labeled-Cu into the cells via DMT-1, suggesting that the Cu^{1+} state was preferred intestinal transporters (Arredondo et al., 2003). However, consistent with other data, the addition of Fe to the culture media inhibited Cu absorption by Caco-2 cells (Arredondo et al. (2003). van Campen and Gross (1968) administered 1 µg of radiolabeled Cu with either 0 or 2.5 mg ascorbic acid to rats via intraduodenal or intraperitoneal injection either simultaneously or delayed by 2 hours. Four treatments involving intraduodenal Cu administration included: 1) control (no ascorbic acid), 2) both labeled-Cu and ascorbic acid given intraduodenally, 3) ascorbic acid injected intraperitoneally at the same time Cu was given intraduodenally, and 4) ascorbic acid was administered 2 hours before Cu was given intraduodenally. An additional treatment group was included in which Cu and ascorbic acid were both given at the same time intraperitoneally. The introduction of both Cu and ascorbic acid intraduodenally decreased the absorption of Cu, while intraperitoneal injection of ascorbic acid (with intraduodenal Cu administration or injection of both Cu and ascorbic acid intraperitoneally) did not alter Cu absorption.

Disilvestro and Harris (1981) evaluated the post-absorptive effect of ascorbic acid on Cu metabolism in chicks. Chicks were raised on either a Cu deficient or Cu adequate diet (n = 56, 8 chicks/treatment). After 12 days of Cu deficiency, chicks received an injection of Cu sulfate (1 mg/kg BW) + saline or L-ascorbic acid (14 mg/kg BW) and were killed 24 h later.

The injection of both Cu sulfate and ascorbic acid to Cu-deficient chicks increased p-phenylenediamine (**PPD**) oxidase (ceruloplasmin) activity (3.27 IU) compared to chicks receiving only Cu-sulfate (1.66 IU). Injection of both Cu and ascorbic acid increased ceruloplasmin values to those comparable with values with Cu-adequate chicks (3.32 IU). Ceruloplasmin is the major Cu transport protein in the body, and the use of PPD to determine activation relates to the oxidation of PPD by ceruolplasmin to yield a colored oxidation product (Sunderman and Nomoto, 1970). The authors suggested that VC may have a role in the movement of Cu from the intestinal cells into tissue enzymes. van den Berg and Beynen (1992) evaluated the impact of 10 g ascorbic acid/kg diet supplemented for six weeks to rats, in which decreases in circulating ceruloplasmin, hemoglobin, and Cu concentrations in the plasma, liver, kidney, heart, spleen, muscle, and bone were observed. In another experiment, van den Berg and Beynen (1992) decreased the ascorbic acid concentration to 1 g ascorbic acid/kg in to rat diets including 1 or 5 mg/kg Cu, and decreased plasma and liver Cu concentrations were still observed.

Antioxidant Interrelationships between Vitamin C, Vitamin E, and Glutathione

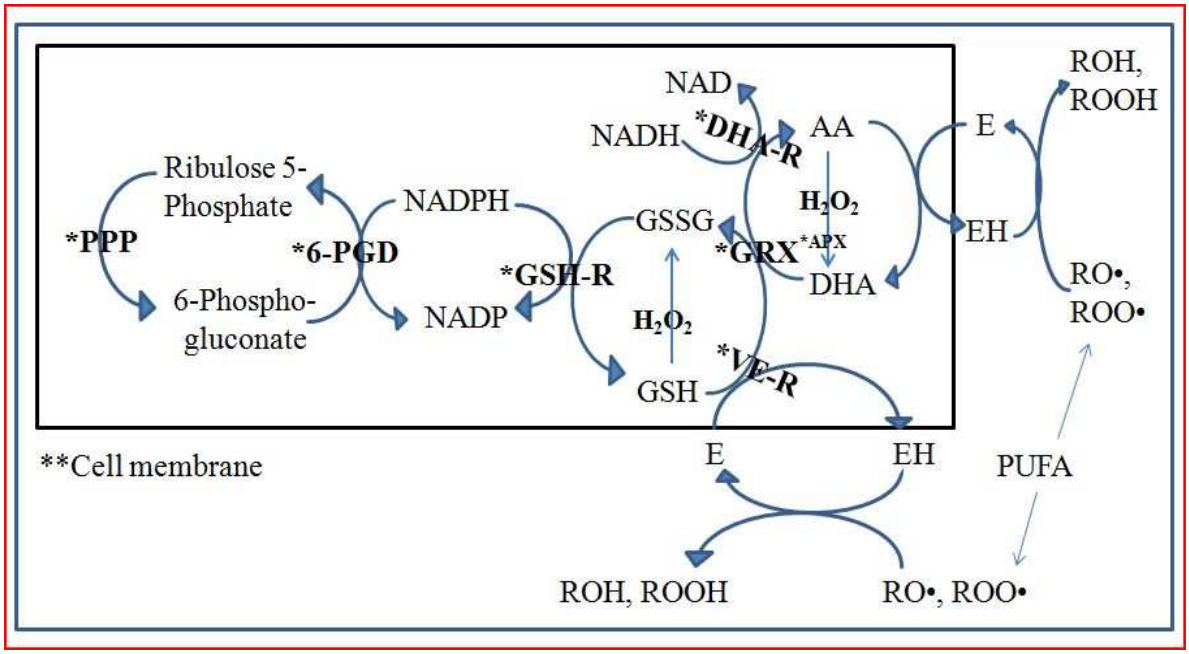
As previously mentioned, in the presence of oxidizing conditions, ascorbic acid is oxidized to DHA via the VC radical, semi-dehydroascorbic acid. Dehydroascorbic acid is immediately transported into cells and reduced to L-ascorbic acid at the expense of GSH or via enzymatic mechanisms (DHA-reductase). Reduction of VC and VE by GSH requires the enzymes glutaredoxin (**GRX**) and VE radical reductase (respectively; Chan, 1993; Rumsey and Levine, 1998). As the cycle continues, GSH may be regenerated to its reduced form by reducing equivalents (NADPH) acquired from the pentose phosphate pathway (Figure 6). In

addition to regeneration by GSH, NAD(P)H can provide necessary electrons to return DHA or semidehydroascorbic acid to ascorbic acid via DHA reductase or semi-dehydroascorbic acid reductase, respectively (Chan, 1993; Rumsey and Levine, 1998).

The relationship between VC and GSH has been well defined. Within adult mice hepatocytes (Mårtensson and Meister, 1992) and bovine aortic endothelial cells (May et al., 2001) the depletion of cellular GSH inhibited the conversion of DHA to ascorbate, suggesting optimal reduction of ascorbic acid occurs in the presence of GSH. Similarly, when GSH is depleted in cells, as a result of oxidative stress, an increase in VC synthesis is observed in mice (Mårtensson and Meister, 1992; Braun et al., 1996; Chan et al., 2004), indicating the compensation of GSH by ascorbate during times of oxidative stress. Chan et al. (2004) suggested that the stimulation of ascorbate synthesis during GSH depletion may be related to increased NAD^+ concentrations of the cell, due to inhibition of coenzyme Q by oxidation, thus triggering the glucuronic acid pathway and production of ascorbic acid. Alternately, the depletion of GSH within the cell may be increasing glycogen metabolism, thus increasing ascorbate synthesis by increased substrate (glucose) availability (Bánhegyi et al., 1997). A possible mechanism by which increased ascorbic acid synthesis, due to GSH depletion in the cell, may be occurring by the inhibition of glycogen phosphorylase phosphatase by GSSG and the subsequent breakdown of glycogen to glucose-1 phosphate, which may be shuttled toward the production of either glucose or ascorbic acid. Glucose-1 phosphate may be converted to UDP-glucose for entry into the glucuronic acid pathway for the synthesis of ascorbic acid.

In dairy cows, Padilla et al. (2006) reported the circulating VC concentrations in heat stressed cows (n = 4; 2 × 2 crossover design, 12 d periods; 18°C vs. 28°C) were

approximately half that of the non-heat stressed cows 1,540 μg/L and 3,120 μg/L, respectively, after 12 d. The authors suggested that the lack of glycogenolysis in heat stressed cows may be altering the availability of glucose for ascorbic acid synthesis, as Braun et al. (1996) identified that the breakdown of glycogen in the liver can stimulate the production of ascorbic acid. Additionally, subjecting animals and plants to heat stress can trigger the production of ROS and may lead to the development of oxidative stress (Zuo et al., 2000; Tiwari et al., 2002; Mujajid et al, 2005). Because antioxidants will be consumed in the presence of oxidants, within the plasma of heat stressed cows and endurance horses a decrease in plasma antioxidant capacity was observed (Harmon et al., 1997; Hargreaves et al. 2002). Interestingly, within the endurance horses, heat stress did not alter the VE content of plasma. This protection of VE suggests the water soluble nature of VC may allow more freedom to regenerate lipid soluble antioxidants.



Adapted from Rumsey and Levine (1998) and Rucker et al. (2008)

Figure 6. The interrelationship between vitamin C, vitamin E, and glutathione. 6-PGD: glucose 6-phosphate dehydrogenase; AA: ascorbic acid; APX: ascorbic acid peroxidase; DHA-R: dehydroascorbate reductase; DHA: dehydroascorbic acid; E: oxidized vitamin E; EH: reduced vitamin E; GSH: reduced glutathione; GSH-R: glutathione reductase; GRX: glutaredoxin; GSSG: oxidized glutathione; H_2O_2: hydrogen peroxide; NADH: nicatinamide adenine dinucleotide; NADPH: nicatinamide adenine dinucleotide phosphate; PPP: pentose phosphate pathway, RO•/ROO•: lipid derived radicals; ROH/ROOH: resolved lipid radicals (reduced lipids); VE-R: vitamin E radical reductase.

Vitamin C and Adipocytes

Development of adipose tissue occurs in "waves" throughout maturity, where cattle begin depositing fat near the kidneys and advance to intermuscular, subcutaneous, and finally intramuscular depots (Owens et al., 1993). Hood and Allen (1973) observed that intramuscular adipose tissue has development patterns different from those of subcutaneous adipose tissue, also suggesting hyperplasia is important for the development of late-developing intramuscular adipose tissue. Additionally, the site and quantity of fat deposition

is a major factor affecting meat quality and efficiency of production (Hood and Allen, 1973). Smith and Crouse (1984) observed that glucose was the preferred substrate for the synthesis of lipids in intramuscular adipocytes compared to acetate in subcutaneous adipocytes.

In cell culture, the differentiation of adipocytes is confirmed by the activity of glycerol-3 phosphate dehydrogenase (**GPDH**) or by the presence of lipid-laden cells determined by Oil-Red-O staining. Glycrol-3 phosphate dehydrogenase activity is measured by the oxidation of NADH in the presence of dihydroxyacetone phosphate (Kozak and Jensen, 1974), as this process is quickly followed by GPDH catalyzing the dephosphorylation of glycerol-3-phosphate into glycerol. A greater GPDH activity results in greater glycerol production for subsequent lipid accumulation, as glycerol comprises the backbone of triglycerides. The use of Oil-Red-O to quantify lipid-laden cells is determined by staining cultured cells with red dye, capturing images of the cells, and enumerating the cells with lipid droplets present in the cytoplasm (Weiser et al., 2009).

A review of the literature supports the hypothesis that VC increases GPDH activity, and therefore the accumulation of lipid within cells. Several authors have evaluated the addition of L-ascorbic acid and L-ascorbic acid-2-phosphate to 3T3-L1 cell lines (a murine adopogenic cell line; Ono et al., 1990; Kawada et al., 1990) and cells cultured from adipose tissue of two Asian breeds of cattle (Japanese black or Hanwoo; Torii et al., 1998; Lee et al., 2000). In response to VC supplementation, these authors observed an increase in GPDH activity and in turn increased lipid accumulation in cells (by Oil-Red-O staining). Among these studies, the addition of VC at concentrations of 10 µmol/L or greater (50, 200, or 500; Kawada et al., 1990; Torii et al., 1998; Lee et al., 2000) produced the greatest increase in GPDH activity and triglyceride accumulation. These values of VC inclusion were selected

because of the nearness to physiological plasma VC concentrations (approximately 5 to 35 µmol/L; 880 to 6,164.2 µg/L) reported by Vavich et al. (1945) and Hidiroglou et al. (1977). Conversely, the inclusion of VC at 1.02 to 1.99 µmol/L to 3T3-L1 cells resulted in a decrease in GPDH activity and lipid accumulation (Hasegawa et al., 2002).

The role of ascorbic acid in adipocyte differentiation was evaluated by Weiser et al. (2009), which was accomplished by utilizing varying doses of ascorbic acid, at 0 to 567.8 µmol/L (0 to 100,000 µg/L) to determine the differentiation of rat bone marrow-derived mesenchymal stromal cells to adipocytes. Ascorbic acid was introduced to the media during propagation and adipogenic periods of growth. Cells incubated with ascorbic acid constantly present in the media from propagation through adipogenesis experienced a greater response of lipid accumulation compared to those receiving ascorbic acid only during propagation or adipogenesis. Furthermore, a dose dependent increase in adipocytes and fraction of adipocytes (percent) was observed. The results of Oil-Red-O staining suggest that supplementing at least 5.68 µmol/L (1000 µg/L) was sufficient to increase the number of adipocytes and amount of lipid accumulation within those cells compared to the controls that receiving no ascorbic acid in the media. This information, while referring to cultured adipocytes, may indicate the supplementation of VC during an earlier time point to cattle diets, such as in creep diets or receiving diets, may potentially provide a means to enhance lipid accumulation later in the finishing period.

Torii et al. (1998) evaluated the adipogenic effects of L-ascorbic acid 2-phoshpate, which was used because of its greater stability compared to ascorbic acid, on freshly isolated adipocytes (perirenal) collected from three, adult Japanese black cattle. The inclusion of L-ascorbic acid 2-phosphate to differentiation media enhanced the GPDH activity and

increased the number of differentiated adipocytes. This increase in activity and number was dose dependent. Specifically greater increases in GPDH activity and cell numbers were observed when 50 µmol/L of L-ascorbic acid 2-phosphate (14,277 µg/L) or greater were included. The 50 µmol/L dose of L-ascorbic acid 2-phosphate is close to the physiological VC concentration of 45 µmol/L (7,925 µg/L) in Japanese black cattle observed by Takahashi et al. (1999), however, this dose is approximately two times greater than the ascorbate concentrations of Japanese black × Holstein cows (2,670 to 4,080 µg/L; 15.2 to 23.2 µmol/L) that were noted by Padilla et al., 2007 and healthy beef cattle (2,395 to 4,702 µg/L; 13.6 to 26.7 µmol/L) reported by Smith et al. (2009). These authors attributed the increase in adipogenic activity to the role of VC in collagen production, as the concentration of protein was not different among treatments, indicating VC did not affect cell division or viability. These authors suggested the manipulation of circulating VC concentration of fattening cattle provides a way to alter lipid accumulation during the finishing phase of the feedlot cattle.

Interestingly, while VC inclusion to cell culture media increased GPHD and lipid-laden cells, Abe et al. (2010) added antioxidant rich kiwi extract (low in VC) to media containing 3T3-L1 cells and noted an increase in GPDH activity and lipid accumulation in cells. An interesting question arises, of whether VC or the availability of antioxidants are responsible for greater GPDH activity and subsequent lipid accumulation?

Vitamin C and glucose uptake by adipocytes

There is limited information available concerning the impact of VC on glucose uptake by adipocytes, and the research available offers opposing results. Glucose is an important substrate for the production of lipids in the adipocytes, and Smith and Crouse (1984)

observed the incorporation of glucose to adipocytes for lipid synthesis was greater in intramuscular adipocytes compared to subcutaneous adipocytes. Abe et al. (2010) reported the inclusion of kiwi extract, as kiwi fruit are known to be excellent sources of VC (contain approximately 105 to 120 mg ascorbic acid/100g of fruit; Vissers et al., 2013), to cultured cells at 30 and 100 mg/L increased basal glucose uptake 1.6 and 1.8 fold, respectively, in adipocytes compared to controls. Additionally, kiwi extract (100 µg/mL) increased insulin-stimulated glucose uptake 1.2-fold in comparision to insulin alone. Conversely, Garcia-Diaz et al. (2010) noted a decrease in glucose uptake by rat adipocytes when VC was included above 50 µM (200 or 1000 µM), which may potentiallybe caused by transporter competition between glucose and VC. These data may indicate the increase in glucose uptake by cells may be related to antioxidant capabilities. No data is available concerning the influence of VC on plasma insulin concentration in feedlot cattle.

Roles of the Extracellular Matrix

The ECM is a complex of glycoproteins, proteoglycans, and glycosaminoglycans that provide a unique and specific environment around cells. While the role of the ECM varies among tissues and needs of the cell, confirmed roles of the ECM include: developing cell structure (shape), cellular migration, spatial development, filtering molecules in contact with the cell, and facilitating interaction of the cell with its environment (Adams and Watt, 1993; Nakajima et al., 2002). The ECM may modulate cell behavior by regulating interactions of molecules with their cellular receptors, specifically growth factors (Adams and Watt, 1993; Nakajima et al., 2002).

Extracellular matrix development and adipocyte differentiation

The ECM may facilitate the rearrangement of adipocyte cytoskeletal components, changing the shape from fibroblastic to spherical, or regulate the interaction of growth factors with their receptors resulting in an intracellular cascade to signal differentiation (Gregorie et al., 1998). Adipocytes are derived from pluripotent stem cells that have the ability to differentiate into mesenchymal precursor cells (multipotent stem cells), and further develop into preadipocytes given appropriate gene expression and growth factors. During terminal differentiation, an increase in the transcription factors CCAAT/enhancer binding protein and peroxisome proliferator-activated receptor-γ have been noted, aiding in the activation of mature adipocyte-specific genes (lipoprotein lipase; GLUT-4, leptin, GPDH; Gregorie et al., 1998; Wu et al., 1996). Nakajima et al (1998) observed that a decrease in type I and III collagen genes (coding for fibril type collagens) during adipocyte differentiation, while type IV collagen genes (coding for network-forming collagen) increased; indicating the unique arrangement of the ECM components is important in the development of specific cell types (Nakajima et al., 1998; Hausman et al., 2009). The network-forming type IV collagen interconnects adipocytes, forming clusters and allowing for adhesion between adipocytes (Aratani and Kitagawa, 1988; Hausman et al., 2009).

Some authors have hypothesized that the role of VC in adipocyte differentiation is through its role in ECM metabolism (Kawada et al., 1990; Torii et al., 1998; Lee et al., 2000). The water soluble properties of VC enable it to exert its greatest activity within the ECM of cells, where its roles include regenerating lipid bound VE and contributing to the hydroxylation reaction of amino acids, specifically lysine and proline, within the pro-collagen peptide chain before exportation to the ECM (Aberle et al., 2001). Ono et al. (1990)

reported the addition of ascorbic acid 2-phosphate stimulated the synthesis and secretion of type IV collagen from both 3T3-L1 preadipocytes and adipocytes. Supplementation of ascorbic acid during the adipogenic phase of rat bone marrow-derived mesenchymal stromal cells increased hydroxyproline accumulation (collagen) in a dose dependent manner with a plateau occurring at 56.8 µmol/L (10,000 µg/L) ascorbic acid, regardless of the availability of ascorbic acid in the media during the proliferation phase (Weiser et al., 2009).

Nakajima et al. (2002) examined the effects of ethyl-3-4-dihydroxybenzoate (**EDHB**), an inhibitor of prolyl hydroxylase and an analog to ascorbate, on essentiality of collagen for adipocyte differentiation. A decrease in accumulated triacylglycerides in adipocytes exposed to EDHB was observed. However, when collagen types V and VI were introduced to the media, adipocytes were able to recover triacylglyceride accumulation by 23 and 31%, respectively. Therefore, the authors suggest collagen types V and VI have an important role in control of adipose tissue development.

Collagen production in the extracellular matrix

Collagen is one of the most abundant proteins in the body, specifically contributing to the extracellular scaffolding for attachment of cells and anchoring other ECM components together. There are currently 27 different types of collagen identified, however, type I, II, IV, V, and XI are the most abundant forms. Collagen is comprised of three alpha-helical polypeptide chains containing a repeating structure of glycine-X-Y, in which X and Y are typically lysine and proline (Kadler et al., 1996). Within the endoplasmic reticulum, the triple helix structure is developed and the posttranslational hydroxylation of of proline and lysine residues by prolyl and lysyl hydroxylases (respectively) occur to form the unique amino

acids of collagen hydroxyproline and hydroxylysine, respectively (Kadler et al., 1996). Because hydroxyproline is exclusively found in collagen, the measure of hydroxyproline can indicate the amount of collagen within a specific tissue (Wu et al., 1981; Sylvestre et al., 2002). Hydroxyproline formation only occurs on non-helical collagen. The formation of these hydroxyl-residues is facilitated by the enzymes prolyl-4-hydroxylase, prolyl-3-hydroxylase, and lysyl hydroxylase, which require Fe and VC as cofactors (Kadler et al., 1996).

Disilvestro and Harris (1981) reported that supplementation of Cu-deficient chicks with L-ascorbic acid did not influence the activity of the Cu and VC dependent lysyl oxidase; however, the addition of Cu sulfate (1 mg/kg) increased the enzyme activity. When L-ascorbic acid and Cu sulfate were administered together lysyl oxidase activity was impaired, suggesting an antagonistic relationship of L-ascorbic acid on Cu availability. Alternately, because VC is water soluble and quickly cleared from the body, the administration of L-ascorbic acid 75 min post supplementation of Cu sulfate recovered lysyl oxidase activity to similar values as observed in the control chicks.

Procollagen is secreted to the ECM by the Golgi apparatus, after which the ECM the end terminus regions are cleaved by collagenases to yield functional collagen helix, which can then be incorporated into collagen fibrils. Within the ECM, collagen cross-linking is facilitated by oxidative deamination of lysine or hydroxylysine residues by the enzyme lysyl oxidase (Cu and VC dependent) within the collagen chain, specifically the non-helical regions at the terminal ends. This crosslink forms a lysine aldehyde (labile) that interconnects two adjacent collagen molecules, facilitating a head to tail linking. These crosslinks are reversible. These crosslinks indicate the quality and stability of the final collagen molecule

(Kjær, 2004). The maturation of collagen fibrils is facilitated by the irreversible transverse cross link formation, which links three collagen fibrils together to form a three dimensional network (Bailey, 1985).

Collagen turnover is facilitated by the activity of collagenases, such as matrix metalloproteinases (specifically the gelatinases MMP-2 and -9), which have a greater optimum pH (7.4) and have specificity to all three collagen peptide chains, or by lysosomal enzymes that have a lower optimum pH (3.5; Bailey, 1985). This family of Zn-dependent endopeptidases can degrade the majority of the ECM components, but is specifically involved in the degradation of interstitial connective tissues and basement membranes (peri/extracellularly located). Because MMP are produced in the dormant form, activation occurs by the removal of a highly conserved pro-peptide sequence (Massova et al., 1998). The catalytic site of MMP contains two Zn ions and at least one Ca ion. One of the Zn ions has been deemed essential for exertion of proteolytic activity; however, the role of the second Zn ion and Ca ions are currently unknown (Massova et al., 1998).

The activity of matrix metalloproteinases may be stimulated by a host of factors including growth factors, cytokines, hormones, structural rearrangement of the ECM, and oxidative stress (Birkedal-Hansen et al., 1993; Kandasamy et al., 2010). The activity of MMP enzymes is specific among collagen type and location (Birkendal-Hansen et al., 1993). One indicator of collagen turnover (plasma/other body fluids) is the measurement of hydroxyproline, as this produt is in the final step in the degradation of collagen (Wu et al., 1981; Sylvestre et al., 2002). The activity of MMP can be hindered by chelation of Zn (removal from the active site of the enzyme) or interaction with tissue inhibitor of

metalloproteinase (TIMP), which blocks activity of fully functional MMP and the proenzyme MMP (specifically gelatinases; Birkendal-Hansen et al., 1993; Massova et al., 1998).

Extracellular matrix and muscle cell formation

In addition to aiding in differentiation of adipocytes, the ECM has a role in the development of muscle fibers, specifically facilitating the migration, proliferation, and differentiation of myoblasts. The role of collagen in muscle cell formation primarily occurs via participation in structural and/or scaffolding roles and transmission of force during contraction. In postnatal muscle growth, the role of collagen is similar, but it is assumed that collagen turnover is greater to facilitate the increase in muscle fiber growth (Bailey, 1985). Within cultured rat thigh skeletal muscle cells (L6), the addition of L-ascorbic acid 2-phosphate (100 μM) may be promoting myogenesis and hypertrophy of myotubes when compared to the untreated cells (Mitsumoto et al., 1994). Because VC is essential for the production of collagen, these authors interrupted collagen synthesis in cultured cells by the addition of EDHB at 200 μM, and prevented the expression of myogenin by myotubes, suggesting the production of collagen is essential for muscle development and/or regeneration.

Role of Vitamin C in the Alteration of Fatty Acid Profile of Beef Adipose Tissue

Within the liver and adipose tissues, cytochrome *b5* and cyanide-sensitive factor act to convert stearoyl CoA and palmitoyl CoA into their corresponding Δ-9-monounsaturated fatty acids. The transfer of electrons to cyanide-sensitive factor is achieved through the use of the intermediate carrier cytochrome *b5*. Oshino et al. (1971) proposed that reducing

equivalents donated by NADH, NADPH, or ascorbate are required for the activation of cyanide-sensitive factor, which in the reduced state acts as an oxygenase for the desaturation of fatty acids. In comparison to the reducing equivalents donated by NADH or NADPH, ascorbate is classified as a weak donor. However, in order to achieve similar reducing power ascorbate needs to be included at ten times the amount as NADH or NADPH.

Oohashi et al. (1999) supplemented Japanese black steers (n = 12) with 50 g VC $g \cdot steer^{-1} \cdot d^{-1}$for the entire finishing period (15 months), the early finishing period (7 months), or the last half of the finishing period (8 months) and compared performance and meat quality to steers that did not receive VC during the finishing period. Vitamin C added to the diet in the early and for the entire finishing period increased monounsaturated fatty acids and polyunsaturated fatty acids, C16:1, C18:1, and C18:2, and decreased saturated fatty acids (C14:0, C16:0, and C18:0) within the intramuscular fat compared to cattle that did not receive any supplemental VC. These data may support the idea of VC providing reducing equivalents for the desaturation of lipids via their corresponding Δ-9-desaturase.

Vitamin C and Color and Lipid Stability

No research data are currently available concerning the impact of VC on protease activity or muscle protein degradation in cattle. However, the impact of VE supplementation to live animals and the subsequent influence of VE on meat quality attributes has been thus far examined (Rowe et al., 2004*a*,*b*), as VE may be stored in lipid depots. The ability of cattle to synthesize VC and its water soluble nature have likely deterred researchers from examining the impact of supplementing live animals with VC and the possible ensuing impact on meat quality. Alternately, the postmortem addition of VC to meat (steaks and

ground beef) has been examined as a means to prolong the reducing environment for greater lipid and color stability.

The jugular infusion of 500 mL of 50% w/v sodium ascorbate (1.7 M) 5 to 10 minutes prior to harvest of ten young heifers (320 kg BW), resulted in meat samples containing 100 to 200 mg ascorbic acid/kg wet tissue (after 48 hours of chill; ascorbic acid determined by 2.6-dichloroendolindophenol titration method) and extended the shelf life of *longissimus* muscle three days compared to the saline injected control cattle, in which no detectable VC was measured in the meat (Hood, 1975). Mitsumoto et al. (1991) collected strip loins from 11 Holstein steers, and to each 2.54 cm steaks either 0 or 0.1 mL solution of 10% VC (L-ascorbic acid sodium salt) was added to every 20 g of meat by coating the surface of the steak. The steaks were placed under retail simulated lighting for 16 days, and the percent of metmyoglobin, oxymyoglobin, and deoxymyoglobin were determined. These authors reported VC treatment to the surface of steaks delayed metmyoglobin formation by three days compared to controls, as steaks treated with VC took nine days to reach 30% metmyoglobin compared to 6 days for controls. Realini et al. (2004) evaluated the impact of a 0.05% v/w addition of VC (sodium ascorbate) to ground beef collected from 18 Angus-Hereford heifers (grain fed, 112 d) and 14 Hereford steers (grass-fed, 135 d). Vitamin C addition to grain-fed ground beef decreased L* (lightness) and increased a* (redness) after 2 and 8 days of retail display. Additionally, VC incorporation into ground beef from grain-fed cattle increased lipid stability after 8 days of display (decreased TBARS), while no impact of VC was noted in grass-fed ground even though grass-fed beef contained a greater percentage of PUFA (4.35%) than grain-fed beef (2.63%). The decreased lipid oxidation in the grass-fed beef may be attributed to a nearly 2-fold decrease in total lipid content compared to grain-fed

beef (11.43 and 24.57%, grass- and grain-fed beef, respectively), and the possibility of a greater antioxidant capacity of cattle due to a greater quantity of antioxidants (such as α-tocopherol) found in the grass.

Giroux et al. (2001) evaluated the effectiveness of ascorbic acid (included at 0.03 to 0.5%) to maintain acceptable ground beef color (L*, a*, and b* values) after exposure to irradiation (0.5 to 4 kGy) during storage. As irradiation treatments (kGy) increased, the incorporation of ascorbic acid was not effective at maintaining a* (redness) values. However, the addition of ascorbic acid at 0.1% increased a* (redness) values of the irradiated patties, regardless of irradiation strength (1 to 4 kGy). Similarly, Nam and Ahn (2003) noted a similar decrease in a* (redness) values in irradiated ground beef (2.5 kGy), but the addition of 0.1% L-ascorbic acid (wt/wt) maintained a* (redness) values for 7 days after irradiation. These two studies suggest the addition of the water-soluble antioxidant VC is capable of maintaining a reducing environment in postmortem muscle to prolong the color stability of beef.

Vitamin C Conclusion

While cattle possess the ability to synthesize VC in the liver, it remains unclear if the quantity of VC produced by feedlot cattle is adequate to meet their needs. Research evaluating VC status of cattle has primarily been conducted in Japanese breeds of cattle, and extreme variability in circulating VC concentrations has thus far been reported. Because Japanese breeds of cattle are on feed for a substantially longer period of time and have a greater propensity to marble in comparison to typical United States feedlot cattle (British or Continental breeds), the VC requirements of the Japanese breeds of cattle may not be

representative of the needs of cattle in the United States. In addition to breed differences, feedlot cattle in the United States may be more likely to encounter growth promotants (such as steroid implants and beta-agonists), variation in diet composition and processing, ethanol-industry co-products (introducing S and fat), and extreme variability in weather (heat and cold stress) compared to the Japanese breeds. These factors may increase the demand for VC, as VC is essential for collagen production to support the extracellular matrix, adipocyte, and bone growth, and role as an antioxidant to protect tissues against ROS formation (stress). It could stand to reason, that in a feedlot situation the supplementation of an exogenous rumen-protected source of VC may increase animal efficiency from the stand point of sparing glucose (the precursor to VC) for utilization in other growth processes and to increase the antioxidant capacity. Therefore, the following studies were designed to address some of the gaps in the current literature. These studies will specifically address the impact of supplementing a rumen-protected VC to steers fed high S finishing diets on growth performance, antioxidant capacity, carcass traits, and meat quality.

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CHAPTER 3.

HIGH DIETARY SULFUR DECREASES THE RETENTION OF COPPER, MANGANESE, AND ZINC IN STEERS

A paper to be submitted to *The Journal of Animal Science*

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ABSTRACT: To examine the effects of dietary S on diet digestibility and apparent mineral absorption and retention, 16 steers [8 ruminally fistulated (368 ± 12 kg BW) and 8 unmodified (388 ± 10 kg BW)] were used in a 2 × 2 factorial design. Steers were paired by modification status and BW and within each of the two consecutive 28 d periods, four pairs of steers were randomly assigned to either a low S (0.24%) or high S (0.68%) pelleted diet. Hay was fed at 5 or 7% of the diet, during period 1 and 2, respectively. Sodium sulfate was used to increase the S content of the high S diet. The low S steers were fed the previous day's intake of their high S counterpart, while high S steers received 110% of previous day's intake. Steers were adapted to individual metabolism stalls for 4 d, stepped up to diet for 7 d, and after high S steers were consuming ad-libitum intake for 7 d (d 14 of period), total urine and feces were collected for 5 d. Feed intake and orts were recorded daily. Dry matter and OM digestibility were determined. Jugular blood was collected before and after each collection period on d 14 and 20, and liver biopsies were collected on d 0 and 29. Macro (Ca, K, Mg, and Na) and micro (Cu, Mn, and Zn) mineral concentrations were determined for

pellets and hay, orts, feces, urine, and plasma and liver samples from each steer via inductively coupled plasma spectrometry. Dry matter intake, DM and OM digestibility, and fecal output were not different ($P \geq 0.11$) due to treatment, while urine volume was greater ($P = 0.01$) in high S steers. A high S diet decreased plasma Cu ($P = 0.04$) and liver Zn ($P = 0.03$) compared to low S steers. No differences ($P \geq 0.20$) were noted among urinary excretion of Cu, Mn, and Zn. Sodium absorption was greater ($P < 0.01$) in the high S steers. Copper, Mn, and Zn retention was lesser ($P \leq 0.01$) in the high S steers. Apparent absorption of Ca, K, and Mg was not affected ($P \geq 0.18$) by dietary treatment, while absorption of Cu, Mn, and Zn within the high S treatment was lesser ($P \leq 0.06$). In conclusion, consumption of a high S diet for 28 d had limited effects on macro mineral absorption and retention, but decreased trace mineral retention, which may limit growth and production of cattle consuming a high S diet long-term.

INTRODUCTION

Ethanol co-product inclusion to cattle diets provides producers with an economic and nutritional alternative to corn-based finishing diets. However, the variability in the S content (0.3 to greater than 1.0% S; Kim et al., 2012) of co-products poses a risk for performance and health of feedlot cattle. Also, in some areas of the United States high sulfate drinking water can introduce greater quantities of S. Currently, the NRC (2005) recommends 0.15% S in cattle diets. However, maximum tolerable limits currently range from 0.3 to 0.5% S for diets containing less than 15% forage or at least 40% forage, respectively. This range has been set to avoid the deleterious effects of high S diets on health and feedlot and carcass-based performance (Kandylis, 1984; Spears et al., 2011; Uwituze et al., 2011).

An underlying component to successful cattle performance in the feedyard is adequate mineral nutrition, as minerals play critical roles in nerve conduction, cell signaling, and energy metabolism, often acting as enzyme cofactors among other biological roles (Underwood and Suttle, 1991). Sulfur is necessary in cattle diets, as S is a component of the S-containing amino acids, B-vitamins, and sulfhydryl bonds for some enzymes (Suttle, 2010). However, the mineral status of cattle fed a high S diet may be compromised, as increased dietary S may antagonize Cu, Se, Zn, Ca, and Mg, thus limiting their absorption, availability, and use by the animal (Shrift, 1954; Suttle, 1974; Spears et al., 1985; Suttle, 1991). While the negative effects of high S diets on performance, health, and carcass characteristics have been investigated, research data concerning macro and trace mineral retention of cattle consuming a high S diet are limited. Therefore, the objective of the present study was to determine the effect of dietary S on mineral status and the apparent digestibility, absorption, and retention of minerals in steers consuming either a low (0.24%) or high (0.68%) S pelleted diet.

MATERIALS and METHODS

Procedures and protocols for cattle experiments were approved by the Iowa State University Institutional Animal Care and Use Committee (8-09-6796-B).

Animals and Experimental Design

This study was conducted in conjunction with a diagnostic study designed to induce polioencephalomalacia (**PEM**), which is further described by Drewnoski et al. (2012). Sixteen Angus-cross steers were used in a 2 × 2 factorial design to determine the effect of

dietary S on diet digestibility and apparent mineral absorption and retention. Eight of the 16 steers were fitted with a rumen fistula (368 ± 12 kg BW) while the other eight remained unmodified (388 ± 10 kg BW). The steers were paired by their modification status and BW, and steers within each pair were randomly assigned to either a low S (**low S**; 0.24%) or high S (**high S**; 0.68%) pelleted diet (Table 1). The diets were pelleted by a commercial feed mill in Creston, IA. This study consisted of two consecutive 28 d periods, in which eight steers, four pairs of fistulated and unmodified steers, were adapted to individual metabolism stalls [213.4 (l) × 182.9 (h) × 91.4 (w) cm] for 4 d while consuming the low S diet. Following stall adaptation, steers were stepped up to ad-libitum intake for a 7 d period, during which steers received 1% of BW (DM) on d 1 and increased by 0.25% BW over each of the following six d. Starting on d 8, steers consuming the high S diet were fed 110% of the previous day's intake, while the low S steers were fed the previous day's intake of their high S counterpart. Steers were fed once daily; at 0800, hay was fed and at 0815, pellets were fed. Hay was offered to steers at 5% (period 1) of their previous day's pellet intake to decrease the likelihood of acidosis and this was increased to 7% in period 2 because rumen pH was observed to be very low during period 1 (Drewnoski et al., 2012). An automatic cup waterer was available in each stall to allow the steers ad libitium access to water. On d 14, after 7 d of ad libitum feed consumption by the high S steers, the 5 d collection period of total urine and feces was initiated. Prior to and at the completion (d 14 and 18) of collection steers were removed from their stalls so the stalls could be thoroughly cleaned.

During each period, a composite of hay and a composite of pellets were made, composed of daily samples of hay and pellets, and at the conclusion of each period samples were dried in a convection oven at 70°C for 48 h. On a daily basis, the feed offered (pellet

and hay) and feed refused (orts; pellet and hay) for each steer were recorded. Samples of feed offered (pellet and hay) and feed refused (orts; pellet and hay) were collected daily, and dried at the end of each period (feed offered) or daily (feed refused) in a convection oven at 70°C for 48 h. Dry matter intake was calculated by multiplying the percent DM of the appropriate feed and ort sample for each steer by the daily as-fed feed offered and the daily ort values collected for each steer and subtracting orts (DM basis) from feed offered (DM basis).

Prior to the initiation of the collection period, all plastic containers for fecal and urine collection were acid-washed for 4 h in 10% hydrochloric acid, rinsed three times with de-ionized water, and allowed to dry thoroughly before use. Total urine output was collected daily into 20 L plastic carboys that contained 6 M trace metal grade acetic acid and de-ionized water to prevent crystallization and volatilization of urine N. Daily during the collection period, prior to removing a 1% aliquot of urine, urine pH was determined and additional acetic acid was included to achieve a pH of ≤ 5. The 1% urine aliquot was composited in a 2 L plastic container over the 5 d collection period. Urine samples were frozen at -20°C until further analysis. Total fecal output was collected on a tared piece of plastic sheeting (122 × 155 cm) and weighed daily. A 3% fecal aliquot was collected daily, composited, and dried in a convection oven at 70°C for 48 h. Fecal DM was determined by multiplying the percent DM of the appropriate fecal sample for each steer by the total fecal sample collected.

Dry matter and OM of feed, orts, and fecal samples was determined. Approximately 1.0 g of each sample was measured into a glass beaker and dried for 48 h at 105°C, samples were cooled in a desiccator for 2 h and the weight of the dried sample was recorded. The dried sample was ashed in a muffle furnace (630°C for 6 h), samples were cooled in a

desiccator for 2 h and the weight of the ashed sample was recorded. Organic matter intake was calculated by multiplying the percent OM of the appropriate feed or ort sample for each steer by the 105°C DM adjusted value for that sample. Fecal OM was determined by multiplying the percent OM of the appropriate fecal sample by the 105°C DM adjusted total fecal sample collected. Digestibility, both DM and OM, was calculated by subtracting the DM or OM adjusted total fecal output from the DM or OM adjusted total intake (pellets and hay minus pellets and hay orts), dividing the DM or OM adjusted total intake, and subtracting the value from 100.

Jugular blood for plasma mineral analysis was collected in trace mineral grade potassium EDTA vacuum tubes (7 mL/steer; Becton, Dickinson, and Company, Franklin Lakes, NJ) from all steers (n = 16) prior to the start of and after the collection period (on d 14 and 20). Samples were centrifuged at 1,000 × g for 10 min at 4°C and plasma was removed and stored at -20°C until further analysis. Liver biopsies were conducted using the method of Engle and Spears (2000) on d 0 and 27. Biopsy samples were transported on ice to the laboratory and frozen at -20°C until further analysis. Liver samples were dried in a forced air oven at 70°C for 48 h prior to acid digestion.

Fecal, hay, feed, and ort samples were acid digested prior to mineral analysis according to the method described by Richter et al. (2012), while the liver and plasma samples were digested according to Pogge and Hansen (2013). Urine and fecal samples were diluted 1:60 and 1:9, respectively, in 1% nitric acid for the analysis of Ca, K, Mg, and Na. Urine samples were diluted 1:2 with 2% nitric acid for the analysis of Cu, Mn, and Zn. No additional dilutions were required for the analysis of feed and orts macro or trace minerals, or fecal trace minerals. Mineral content was analyzed using inductively coupled plasma optical

emission spectrometry (**ICP**; Optima 7000 DV, Perkin Elmer, Waltham, MA). A bovine liver standard from the National Institute of Standards and Technology (Gaithersburg, MA) was included with each run to verify instrument accuracy and Yttrium (Inorganic Ventures, Christiansburg, VA) was added as an internal standard to all samples to account for variation in sample introduction.

Total mineral content of individual components (feed offered and refused, feces, and urine) was calculated by multiplying the respective value determined via ICP (mg/kg or mg/L) by the total quantity of the feed offered or refused, total fecal output, or total urine output volume. Daily mineral content of mineral intake (pellets and hay), refused pellets and hay, fecal output, and urine output were determined by dividing total mineral content of the respective components by the number of days of collection. Mineral retention was calculated by subtracting mineral excreted from mineral consumed for each steer. The percent mineral retained was calculated by dividing the daily mineral retained by the mineral consumed, multiplied by 100. The percent of apparent mineral absorption was calculated by subtracting fecal mineral from consumed mineral, divided by consumed mineral, and multiplying by 100. The mineral S is not reported, due to the inability to account for the amount of S exiting the body as hydrogen sulfide during eructation.

One steer receiving the high S diet in period 2 was removed from the study after being diagnosed with PEM on d 2 of the collection period (d 17 of the period). Final plasma and liver biopsy samples were collected from that steer (on d 17 of the period) prior to his removal from the study. Following the collection period, the low S counterpart to the steer that developed PEM was removed from study.

Statistical Analysis

Data were analyzed by ANOVA using the Mixed Procedure of SAS (SAS Institute Inc., Cary, NC). The model for the analysis of DM and OM digestibility, plasma and liver mineral, and mineral intake, excretion, and retention data included the fixed effects of treatment, period, and the interactions. Daily DMI and fecal and urine output were analyzed as repeated measures and included the fixed effects of treatment, day, period, and the interactions. Modification status of the steers was tested as a fixed effect, but was not significant and was removed from the model. Day was the repeated effect and steer was the experimental unit for all analyses (n = 16). Initial plasma (d 14) and initial (d 0) liver mineral concentrations were tested as covariates for their respective final mineral concentrations, but were removed from the model when $P \geq 0.2$. Based on the Akaike Information Corrected Criterion, autoregressive 1 was selected as the covariance structure for all repeated measures analysis. Cook's D was used to identify and remove outliers. Significance was declared at $P \leq 0.05$ and tendencies were declared from $P = 0.06$ to 0.10. The values reported in the tables are least squares means.

RESULTS

Dry Matter Intake, Diet Digestibility, and Fecal and Urine Output

Dry matter intake, diet digestibility, and fecal and urine output data are reported in Table 2. Because steers were pair fed, DMI was not influenced ($P = 0.41$) by S content of the diet; however, a period effect ($P = 0.002$) was noted, in which the steers in period 1 consumed approximately 1.07 kg/d more than period 2 steers. Dry matter intake displayed no interactions between treatment, period, or day ($P \geq 0.26$). The DM digestibility ($P = 0.18$),

OM digestibility ($P = 0.54$), and fecal output ($P = 0.11$) were not different as a result of treatment, and no treatment by day interactions ($P \geq 0.20$) were noted for these data. Daily urine output was greater ($P = 0.01$) when steers consumed the high S diet compared to the low S diet, and a tendency ($P = 0.07$) for a treatment by day interaction was observed. This interaction is primarily being driven by the high S treatment, where steers had greater urine output ($P \leq 0.01$) between d 2 and 3, and 4 and 5. Alternately, the low S treatment tended ($P = 0.09$) to decrease urine output between d 1 and 2, but output was not different ($P \geq 0.42$) throughout the remainder of the collection period.

Final Plasma and Liver Mineral Concentrations

Final plasma and liver mineral data are reported in Table 3. Day 14 plasma Cu was lesser ($P = 0.05$) in the high S steers than the low S steers, while no differences ($P \geq 0.39$) were noted among plasma Mg or Zn. On d 20, the high S steers had lesser ($P = 0.04$) plasma Cu concentrations compared to low S steers, while no differences ($P \geq 0.47$) were noted among plasma Mg or Zn concentrations. Day 0 liver concentrations of Cu, Mn, or Zn ($P \geq 0.47$) were not different as a result of treatment; however, the high S steers displayed a lesser ($P = 0.03$) concentration of liver Zn than the low S steers, and no differences ($P \geq 0.34$) in liver Cu or Mn concentrations were noted between the two treatments.

Daily Macro Mineral Intake, Excretion, and Retention

Macro mineral intake, excretion, and retention data are reported in Tables 4 (amount, g/d) and 5 (percent). Because S was introduced to the diet as sodium sulfate the concentration of Na consumed was greater ($P < 0.01$) in the high S treatment than the low S steers;

however, steers in period 2 tended ($P = 0.06$) to consume less Na than steers in period 1. Magnesium intake tended to decrease ($P = 0.09$) with the greater S concentration in the diet, while Ca and K were not different ($P \geq 0.15$) due to S treatment. No treatment by period effect ($P \geq 0.25$) was observed for Ca, Mg, or K intake.

The amount of fecal Na (g/d) was greater ($P = 0.04$), while fecal Ca (g/d) and K (g/d) and lesser ($P \leq 0.08$) in the high S steers than the low S steers. Treatment did not affect ($P \geq 0.50$) fecal amounts of Mg (g/d). However, the amount of fecal Ca, K, and Mg that were excreted (g/d) displayed a period effect ($P \leq 0.07$), in which steers in period 1 excreted more mineral (Ca, K, and Mg, g/d) than period 2. Urinary excretion of Na (g/d) was greater ($P < 0.01$) in steers consuming the high S diet than the low S, and Na (g/d) excretion was greater ($P = 0.02$) in period 1 compared to period 2. The concentration of dietary S did not influence ($P \geq 0.22$) the urinary excretion (g/d) of Ca, K, or Mg; however, period 1 steers had a greater excretion (g/d; $P \leq 0.08$) of K and Mg compared to period 2 steers. No treatment by day ($P \geq 0.35$) interaction was observed for Ca, K, or Mg urine excretion (g/d).

As a percentage of intake (%), fecal excretion Na was lesser ($P = 0.002$) in the high S steers than the low S steers. Fecal excretion (as a percent of intake) of Ca, K, and Mg were greater ($P \leq 0.05$) in period 1 steers compared to period 2. A treatment by period effect ($P = 0.05$) was noted among fecal Ca excretion (%), which is primarily being driven by greater ($P = 0.01$) fecal Ca excretion by the high steers in period 1 than those in period 2. A treatment by period effect ($P = 0.03$) was observed for fecal Mg excretion (%), which is being driven by a tendency for greater ($P = 0.07$) fecal Mg excretion by the high S period 1 steers than period 2 steers. Urinary excretion of Na (%) was greater ($P = 0.001$) in high S steers compared to low S steers.

Steers consuming the high S diet tended to retain a greater amount ($P = 0.07$) of Na (g/d); however, when expressed as a percent of their daily Na intake they actually retained less ($P < 0.01$) Na than the low S diet. The high S diet did not influence the amount of mineral retained (g/d; $P \geq 0.11$) or percent ($P \geq 0.16$) retention of Ca, K, or Mg. Steers in period 1, regardless of S concentration of the diet, retained a lesser ($P \leq 0.07$) amount of Ca (g/d, percent retention, and percent apparent absorption) compared to steers in period 2, likely attributed to lesser fecal excretion of Ca in period 2. However, no effects of period were observed among the amount (g/d; $P \geq 0.48$) or percent ($P \geq 0.11$) of K, Mg, or Na retention. A treatment by period effect ($P = 0.03$) was observed in the percent Na retention. This interaction was likely driven by the greater ($P = 0.01$) percent Na retention of the low S period 1 steers than the low S steers in period 2, while no difference ($P = 0.58$) was noted between the high S treatments of period 1 and 2. The percent absorption of Na was greater ($P < 0.01$) in the high S steers compared to the low S steers, while dietary treatment did not effect ($P = 0.18$) the percent apparent absorption of Ca, K, or Mg. Steers in period 1, regardless of dietary S concentration, had lesser ($P < 0.01$) apparent absorption of K and greater ($P = 0.05$) absorption of Na. The apparent absorption of Mg displayed a treatment by period effect ($P = 0.03$), which was likely a result of greater absorption ($P = 0.02$) of Mg by the low S steers in period 1 than the period 2 steers, while Mg absorption in the high S treatment tended to be lesser ($P = 0.07$) in period 1 steers compared to period 2 steers. A treatment by period effect ($P = 0.05$) was also noted among apparent absorption of Ca, which is being driven by the lesser absorption of Ca by the high S period 1 steers than the high S period 2 steers.

Daily Trace Mineral Intake, Excretion, and Retention

Trace mineral intake, excretion, and retention data are reported in Table 6 (amount, mg/d) and 7 (percent). The intake of Mn and Zn was lesser ($P \leq 0.05$) in steers consuming the high S diet than the low S diet, as the high S diet contained lesser concentrations of these minerals. Steers in period 1, regardless of dietary S concentration, consumed greater amounts (mg/d) of Cu and Mn compared to period 2 steers. A treatment by period effect was observed for the intake of Mn ($P = 0.04$) and Zn ($P = 0.04$).

Fecal Cu (mg/d) and Mn (mg/d) were greater ($P < 0.01$) in steers consuming the high S diet, while fecal Zn (mg/d) was not affected ($P = 0.68$) by dietary treatment. However, steers in period 1 excreted a greater ($P < 0.01$) amount of Cu, Mn, and Zn (mg/d) in the feces compared to period 2 steers. Additionally, there was a treatment by period effect for fecal Cu and Zn ($P < 0.01$), which is related to lesser ($P \leq 0.02$) fecal Cu and Zn excretion (mg/d) by the low S period 1 steers compared to the low S period 2 steers. Urinary excretion (mg/d) of Cu, Mn, and Zn were not different as a result of dietary treatment ($P \geq 0.20$) and Cu (mg/d) and Mn (mg/d) were not different due to period ($P \geq 0.62$); however, urinary Zn excretion (mg/d) was greater ($P = 0.04$) amongst steers in period 1 compared to period 2. No treatment by period interaction was observed ($P \geq 0.28$) for the urinary excretion of the three trace minerals.

When trace minerals are examined on a percentage of total intake (%), fecal excretion of Cu, Mn, and Zn were greater ($P \leq 0.06$) in the high S steers than the low S steers. Urinary excretion of Mn (%) was greater ($P = 0.05$) in the high S steers compared to the low S steers, while urinary Zn excretion (%) tended to be greater ($P = 0.07$) in period 1 steers than period 2 steers.

The consumption of a high S diet decreased the amount (mg/d) of retained ($P < 0.01$), the percent (%) retention ($P \leq 0.06$), and the apparent absorption ($P \leq 0.06$) of Cu, Mn, and Zn compared to steers consuming the low S diet. The amount of Cu, Mn, and Zn retained were not influenced ($P \geq 0.14$) by period. No treatment by period effects ($P \geq 0.10$) were noted among mineral retention (mg/d or percent) and apparent absorption for any of the trace minerals.

DISCUSSION

While ethanol co-products are attractive in price and nutrient profile as feedstuffs for finishing cattle, a greater inclusion in diets may increase the S content of the diet and increase the risk of negatively affecting cattle performance and health (Kandylis, 1984; Spears et al., 2011; Uwituze et al., 2011; Richter et al., 2012). In the present study, DMI of steers was not different due to treatment as steers were pair fed in order to maintain similar intakes between the low and high S steers. However, in the feedyard the impact of high S diets on DMI may vary. It is important to note the dietary S concentration of 0.68% used in the present study was designed to induce PEM and is less likely to be experienced in a typical feedlot diet.

Dietary S exceeding 0.20% has been well documented to decrease DMI of growing goats (Qi et al., 1993a), Holstein steers (Zinn et al., 1997), and feedlot steers (Spears et al., 2011; Uwituze et al., 2011). Because steers in the present study were pair-fed DMI was not different, which may help explain the lack of differences observed among diet digestibility (DM and OM) between the high and low S steers. Similar to the present study, when DMI was similar between treatments, total tract digestibility was not altered by the consumption of

either 17.8 or 32.9 g S/d (Robertson et al., 1996) or dietary S concentrations of 0.15 to 0.25% (Zinn et al., 1997). Alternately, Uwituze et al. (2011) reported that steers consuming a 0.65% S diet consumed less DM (approximately 0.7 kg DM/d) and increased DM and OM apparent digestibility than the steers consuming a 0.42% S diet, which may indicate that rate of passage is contributing to diet digestibility.

The formation of Cu-sulfide is a notable and well-studied antagonism of S in the ruminant (Suttle, 1991; Spears, 2003). Furthermore, Mo in the rumen or circulation results in the scavenging of Cu by the S-Mo compound thiomolybdate, which may be found in multiple forms such as di-, tri-, and tetra-thiomolybdates, wich may be further diminishing the Cu availability for utilization in biochemical processes (Suttle, 1991; Gould and Kendall, 2011). Because the antagonism between Cu and Mo results in rapid depletion of Cu status of the ruminant, it is recommended that for every 1 mg Mo/kg diet, the concentration of available Cu should be increased by 8 mg Cu/kg diet (Marston, 1999). Kelleher et al. (1983) showed that radiolabeled tri- and tetra-thiomolybdate were absorbed from the rumen of sheep, and gained entry into circulation. In the present study, Cu intakes were not different as a result of S treatment, but an increase in fecal Cu (mg/d) resulted in lesser apparent absorption of Cu by the high S steers. The greater amount of Cu lost may be attributed to the inability of Cu-sulfide or the complex formed between Cu, S, and Mo to be absorbed in the gastrointestinal tract, thus contributing to greater excretion of Cu in the feces (Suttle, 1991). Additionally, Gooneratne et al. (1994; 2011) reported increasing concentrations of both dietary S and Mo increased both biliary and urinary excretion of Cu in Angus and Simmental heifers after 2 mo of diet consumption. Copper is primarily excreted in the bile, which ultimately contributes to fecal losses of Cu.

Consuming a high S diet for 28 d decreased plasma Cu approximately 34% compared to the low S steers. Others have reported a similar decrease in circulating Cu concentrations when cattle consumed a high S diet for 76 d (Suttle, 1974), 149 d (Pogge and Hansen, 2013), or 155 d (Richter et al., 2012). Alternately, when S is consumed for a similar time period (28 d) as the present study, no differences in serum Cu concentration were noted in steers consuming S-fertilized tall-fescue (3.3 or 4.0 mg/g for control and fertilized, respectively) or orchardgrass (2.9 or 3.7 mg/g for control and fertilized, respectively; Spears et al., 1985) or Angora goats consuming 0.16 and 0.34% S diets (Qi et al., 1993b). Contrary to plasma, liver Cu concentration was not affected by the 28 d consumption of a high S diet in the present study. Arthington et al. (2002) similarly reported no difference in liver Cu concentration of cows consuming S-fertilized grasses over a 117 d period, while others have observed a decrease in liver Cu caused by greater S content of the diet [Bremner and Young, 1978 (210 d study); van Ryssen et al., 1998 (74 d study); Spears et al., 2011 (116 d study); Richter et al., 2012 (155 d study); Pogge and Hansen, 2013 (149 d study)]. However, these contradictory results may be caused by the number of days consuming a high S diet, as the current study (28 d) may not have been of sufficient length for cattle to deplete liver Cu stores.

In contrast to the well-defined S-Cu interaction, little information is known about how S may impact Mn and Zn absorption and retention. While plasma and liver Zn are often used as biomarkers of Zn status, Hambidge (2003) indicated that because the pools of Zn in the body undergo constant exchange, no ideal biomarker of Zn status has been identified. Plasma Zn was not altered because of dietary S concentration in the current study. These data concur with findings reported in steers (Spears et al., 1985), goats (Qi et al., 1993b), and sheep

(Smith and White, 1997). Increasing the S content of the diet decreased liver Zn in the present study, while others have reported an increase in liver Zn of lambs consuming 0.35% S compared to those consuming 0.18% S (Felix et al., 2012) or no difference in liver Zn concentration of sheep (Smith and White, 1997), steers (Richter et al., 2012), or cows (Arthington et al., 2002) consuming increased dietary S. Similar to Felix et al. (2012) and Pogge and Hansen (2013), liver Mn concentration was not different as a result of dietary S content in the present study. The lack of S effect observed on liver Cu and Mn concentrations might be related to the short period of time that cattle received a high S diet. The percent retention and apparent absorption of Mn and Zn were approximately 3-fold less in the high S steers compared to the low S steers, and as far as the authors are aware, this paper is the first to report a decreased retention of Mn and Zn in cattle consuming a high S diet. It is our hypothesis that the complexes Mn- and Zn-sulfide are being formed within the rumen thus limiting availability for absorption, as Mn- and Zn-sulfide formation can occur in biological situations (Rickard and Luther, 2006). This possible interaction of S and Mn or Zn may help explain the greater percentage of consumed Mn and Zn that were excreted in the feces of the steers consuming a high S diet. However, further research is necessary to elucidate the impact of S on these divalent metals and the subsequent impact on cattle performance.

In contrast to the trace minerals, dietary S had limited impacts on the macro mineral balance of steers. The use of sodium sulfate to increase the concentration of S in the diet is likely a contributing factor to the greater Na intake and urinary excretion as a percent of total intake by the high S steers. Similarly, the consumption of high sulfate coal-pit water by steers increased Na excretion approximately 4-fold (Robertson et al., 1996), even though water consumption and urine volume were not different. Robertson et al. (1996) suggested that

greater Na excretion might be related to overall greater solute excretion in the urine to maintain osmotic balance. The urine volume of the high S steers in the present study was approximately 3.4 L more/day compared to low S steers, which may relate to an increased clearance of both Na and S (as urinary sulfate; Underwood and Suttle, 1991). The greater urine volume may also be caused by greater water intake; however, individual water intake of steers was not monitored in the present study. Neville et al. (2011) reported that as the concentration of dietary S increased from 0.22 to 0.84% S, by increasing DDGS inclusion rates, lambs linearly increased daily water consumption and excreted more S in the urine. However, our experimental design did not allow us to conclude whether the increased urine output by high S steers was a function of increased dietary S, or Na from the use of sodium sulfate.

Circulating Mg concentrations were not different as a result of treatment in the present study. Richter et al. (2012) and Pogge and Hansen (2013) also reported no difference in plasma Mg when steers consuming diets containing 0.22 to 0.6% S finishing diets. While Ca and Mg intake and retention in the present study were not altered by dietary S concentration, Spears et al. (1985) reported the apparent absorption of Ca and Mg of Hereford steers consuming 0.37% forage S from S-fertilized cool season grasses was lesser than the control steers (no S-fertilization). Robertson et al. (1996) reported no difference in Ca retention of steers consuming high sulfate coal-pit water compared to the low sulfate town water.

Because cattle consuming a high S diet can experience a decrease in DMI, thus decreased mineral intake, and reduced mineral absorption due to S antagonisms, the provision of adequate trace minerals to feedlot cattle consuming a high S diet is paramount as

the availability of these minerals for absorption and utilization by the animal is likely compromised. Since minerals play an essential role in immunity and growth, thought should be given to the recommended trace mineral concentrations of cattle consuming a high S diet. The NRC (2000) recommends increasing the supplemented trace minerals stressed or newly received cattle, which often consume less DMI, by approximately two times the recommended concentrations for non-stressed cattle. Similarly, an increase in supplemented mineral in a high S diet may aid in maintaining mineral status in the animal. Additionally, an injectable mineral may limit the depletion of trace minerals by high S diets, as injected minerals bypass the gastrointestinal tract antagonisms. An injectable mineral may also help bolster the trace mineral status of cattle consuming a high S diet, which may slightly lessen the dependence of cattle on gastrointestinal absorption of trace minerals for incorporation into biological processes. When an injectable trace mineral (containing Cu, Se, Mn, and Zn) is used in concert with NRC (1996) recommended trace mineral supplementation, plasma Cu and Se (Pogge et al., 2012) and liver Cu (Daugherty et al., 2002, Pogge et al., 2012) and Se (Pogge et al., 2012) concentrations were increased in cattle. However it is important to note these cattle were not consuming a high S diet.

Sulfur has been identified as a potential contributor toward the development of oxidative stress (Truong et al., 2006; Pogge and Hansen, 2013). The development of oxidative stress is hypothesized to occur via the depletion of the antioxidant glutathione, which is involved in the clearance of excess S from the body, and the disruption of cytochrome *c* oxidase (a Cu dependent enzyme), which results in increased production of reactive oxygen species and reactive S species (Truong et al., 2006). Because high S diets can decrease the trace mineral status of an animal the cellular antioxidants superoxide

dismutase (Cu, Mn, Zn dependent) and glutathione peroxidase (Se dependent) may be unable to efficiently remove reactive oxygen species and reactive S species, thus further contributing to oxidative stress.

In conclusion, the consumption of high S diets decreased the retention of Cu, Mn, and Zn and had limited impacts on the macro minerals, Ca, K, Mg, and Na. These results suggest a long-term exposure of feedlot cattle to high S diets may require additional trace mineral supplementation to ensure the maintenance of adequate mineral nutrition for optimal growth and production.

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Table 1. Ingredient composition of diet (DM basis)

	% DM[1]	
Item	Low S	High S
Wheat middlings	40.8	40.1
Corn dried distiller's grains plus solubles	25.4	24.7
Corn starch	23.1	22.4
Molasses	3.9	3.9
Cottonseed hulls	3.7	3.7
Sodium sulfate	--	2.0
Limestone	1.7	1.7
Trace minerals and vitamin A[1]	0.1	0.1
Analyzed mineral content of the diet		
Ca, %	0.86	0.87
K, %	1.07	1.03
Na, %	0.14	0.70
Mg, %	0.34	0.33
S, %	0.24	0.68
Cu, mg/kg	15.5	17.0
Mn, mg/kg	124.7	96.6
Zn, mg/kg	119.3	109.2

[1] Trace minerals (Co, Cu, I, Mn, Se, and Zn) were added to provide 100% of the requirement based on NRC (1996) NRC and vitamin A was included at 4,400 IU/kg of diet DM

Table 2. Influence of dietary S concentration on daily dry matter intake, diet digestibility, and daily fecal and urine output of steers fed a low sulfur (0.24% S) or high sulfur (0.68% S) diet

Dietary treatment	Period 1		Period 2					
	Low S	High S	Low S	High S	SEM	*P* Value		
Steers (*n*)	4	4	4	4		Trt[1]	Prd[2]	Trt*prd[3]
DM intake[4], g/d	6.28	6.18	5.34	4.99	0.200	0.41	0.002	0.67
DM digestibility[5], %	71.66	71.72	69.03	73.02	1.437	0.18	0.65	0.20
OM digestibility[6], %	69.47	68.74	66.75	69.71	1.792	0.54	0.63	0.32
Daily output								
Fecal[7], kg DM/d	1.78	1.77	1.65	1.31	0.106	0.11	0.001	0.12
Urine[8], L/d	5.04	10.67	6.54	7.73	1.215	0.01	0.53	0.07

[1]Treatment
[2]Period
[3]Treatment by period
[4]Daily dry matter intake, repeated measures averages: day ($P = 0.08$); treatment by day ($P = 0.26$); treatment by day by period ($P = 0.80$)
[5]Dry matter digestibility
[6]Organic matter digestibility
[7]Fecal dry matter output, repeated measures: day ($P = 0.001$); treatment by day ($P = 0.21$); treatment by day by period ($P = 0.002$)
[8]Urine output, repeated measures: day ($P = 0.01$); treatment by day ($P = 0.07$); treatment by day by period ($P = 0.12$)

Table 3. Influence of dietary S concentration on final plasma (d 20) and liver (d 27) mineral concentrations of steers fed a low S (0.24% S) or high S (0.68% S) diet

Dietary treatment	Low S	High S	SEM	*P* Value
Steers (*n*)	8	8		
Plasma mineral, d 20[1]				
Cu[2], mg/L	1.06	0.75	0.096	0.04
Mg[2], mg/L	22.32	24.76	2.285	0.47
Zn[2], mg/L	1.13	1.25	0.138	0.57
Initial liver mineral[3]				
Cu[4], mg/kg DM	132.62	143.75	21.257	0.71
Mn[4], mg/kg DM	10.49	10.94	0.431	0.47
Zn[4], mg/kg DM	111.95	109.12	9.001	0.82
Liver mineral[3]				
Cu[4,5], mg/kg DM	181.2	145.8	27.65	0.34
Mn[4], mg/kg DM	12.7	12.3	0.52	0.62
Zn[4], mg/kg DM	114.5	93.6	5.86	0.03

[1]Plasma mineral collected beginning and end of 5 d collection period (d 14 and 20), one high S steers was removed from study d 2 of collection period after developing polioencephalomalacia symptoms

[2]Period: $P \geq 0.15$; treatment by period: $P \geq 0.15$

[3]Liver mineral collected on d 0 and 27 of the study

[4]Period: $P \geq 0.20$; treatment by period: $P \geq 0.14$

[5]Day 0 liver Cu used as a covariate

Table 4. Influence of dietary S concentration on the amount of daily macro mineral intake, fecal and urine excretion, and mineral retention (g/d) of steers fed a low S (0.24% S) or high S (0.68% S) diet

Dietary treatment	Period 1		Period 2					
	Low S	High S	Low S	High S	SEM	*P* Value		
Steers (*n*)	4	4	4	4		Trt[1]	Prd[2]	Trt*prd[3]
Mineral intake								
Ca, g/d	51.43	44.27	47.73	48.79	3.368	0.38	0.91	0.25
K, g/d	69.03	65.13	58.47	50.89	3.752	0.15	<0.01	0.63
Mg, g/d	21.12	19.14	17.83	15.79	1.087	0.09	0.01	0.98
Na, g/d	8.45	42.37	7.01	30.89	2.368	<0.01	0.02	0.06
Fecal excretion								
Ca, g/d	32.79	30.41	30.10	16.99	3.987	0.08	0.07	0.20
K, g/d	9.54	7.49	4.95	3.74	0.710	0.04	<0.01	0.56
Mg, g/d	15.87	17.43	15.09	12.27	0.907	0.50	<0.01	0.03
Na, g/d	1.30	3.99	1.94	3.63	0.938	0.04	0.89	0.61
Urinary excretion								
Ca, g/d	0.23	0.30	0.34	0.35	0.096	0.70	0.39	0.75
K, g/d	42.36	45.29	38.13	34.45	3.411	0.91	0.05	0.35
Mg, g/d	1.58	1.77	0.86	1.40	0.282	0.22	0.08	0.55
Na, g/d	1.21	30.51	2.62	18.84	1.992	<0.01	0.02	<0.01
Mineral retention								
Ca, g/d	18.42	13.55	17.28	31.45	4.287	0.30	0.07	0.05
K, g/d	17.12	12.36	15.39	12.71	4.276	0.40	0.87	0.81
Mg, g/d	3.66	-0.07	1.87	2.12	1.021	0.11	0.85	0.08
Na, g/d	5.93	7.87	2.45	8.40	2.019	0.07	0.48	0.34

[1]Treatment
[2]Period
[3]Treatment by period

Table 5. Influence of dietary S concentration on macro mineral fecal and urine excretion, mineral retention, and mineral absorption as a percent of intake of steers fed a low S (0.24% S) or high S (0.68% S) diet

Dietary treatment	Period 1		Period 2					
	Low S	High S	Low S	High S	SEM	*P* Value		
Steers (*n*)	4	4	4	4		Trt[1]	Prd[2]	Trt*prd[3]
Fecal excretion								
Ca, %	63.62	69.44	62.80	34.59	7.793	0.18	0.04	0.05
K, %	13.84	11.66	8.56	7.30	1.202	0.18	0.002	0.71
Mg, %	75.22	91.41	84.56	79.16	4.336	0.23	0.74	0.03
Na, %	15.50	9.21	27.54	10.69	3.069	0.002	0.05	0.11
Urinary excretion								
Ca, %	0.43	0.69	0.73	0.82	0.238	0.49	0.40	0.74
K, %	61.33	69.10	65.51	71.45	7.070	0.35	0.65	0.90
Mg, %	7.50	9.23	4.83	10.41	2.524	0.17	0.77	0.46
Na, %	14.43	71.65	38.54	64.31	10.088	0.001	0.43	0.14
Mineral retention								
Ca, %	35.94	29.86	36.47	64.59	7.857	0.18	0.05	0.05
K, %	24.82	19.25	25.92	21.24	6.864	0.46	0.83	0.95
Mg, %	17.28	-0.64	10.61	10.43	6.020	0.16	0.72	0.17
Na, %	70.06	18.84	33.91	25.01	8.699	<0.01	0.11	0.03
Apparent absorption								
Ca, %	36.37	30.56	37.20	65.41	7.793	0.18	0.04	0.05
K, %	86.16	88.34	91.43	92.69	1.203	0.18	<0.01	0.71
Mg, %	24.78	8.59	15.44	20.84	4.336	0.23	0.74	0.03
Na, %	84.50	90.79	72.45	89.31	3.070	<0.01	0.05	0.11

[1]Treatment
[2]Period
[3]Treatment by period

Table 6. Influence of dietary S concentration on the amount of daily micro mineral intake, fecal and urine excretion, and mineral retention of steers fed a low S (0.24% S) or high S (0.68% S) diet

Dietary treatment	Period 1		Period 2					
	Low S	High S	Low S	High S	SEM	*P* Value		
Steers (*n*)	4	4	4	4		Trt[1]	Prd[2]	Trt*prd[3]
Mineral intake								
Cu, mg/d	90.93	104.98	84.74	79.90	5.182	0.39	0.01	0.09
Mn, mg/d	715.85	594.94	684.12	415.71	32.439	<0.01	<0.01	0.04
Zn, mg/d	638.94	642.22	676.86	510.78	37.383	0.05	0.23	0.04
Fecal excretion								
Cu, mg/d	68.11	105.05	53.20	66.02	4.602	<0.01	<0.01	<0.01
Mn, mg/d	597.15	558.74	553.00	83.96	32.580	<0.01	<0.01	0.07
Zn, mg/d	550.90	663.61	551.79	464.58	30.341	0.68	<0.01	<0.01
Urinary excretion								
Cu, mg/d	0.24	0.39	0.30	0.32	0.057	0.20	0.96	0.28
Mn, mg/d	0.43	0.61	0.38	0.52	0.130	0.24	0.62	0.88
Zn, mg/d	2.19	2.68	1.61	0.92	0.507	0.85	0.04	0.27
Mineral retention								
Cu, mg/d	21.98	0.27	21.14	13.67	3.934	<0.01	0.14	0.10
Mn, mg/d	117.61	37.27	133.22	34.23	28.354	<0.01	0.83	0.75
Zn, mg/d	86.62	-17.39	126.26	49.83	25.652	<0.01	0.06	0.60

[1]Treatment
[2]Period
[3]Treatment by period

Table 7. Influence of dietary S concentration on daily micro mineral fecal and urine excretion, mineral retention, and apparent mineral absorption as a percent of intake of steers fed a low S (0.24% S) or high S (0.68% S) diet

Dietary treatment	Period 1		Period 2					
	Low S	High S	Low S	High S	SEM	*P* Value		
Steers (*n*)	4	4	4	4		Trt[1]	Prd[2]	Trt*prd[3]
Fecal excretion								
Cu, %	74.93	99.80	74.51	84.96	4.792	0.003	0.14	0.56
Mn, %	83.42	93.97	80.70	95.86	6.237	0.06	0.95	0.72
Zn, %	86.24	103.41	81.30	94.26	5.544	0.02	0.23	0.71
Urinary excretion								
Cu, %	0.27	0.36	0.36	0.42	0.068	0.30	0.29	0.75
Mn, %	0.06	0.10	0.06	0.14	0.028	0.05	0.55	0.48
Zn, %	0.34	0.41	0.24	0.18	0.081	0.99	0.07	0.41
Mineral retention								
Cu, %	24.81	-0.16	25.13	14.63	4.805	<0.01	0.14	0.16
Mn, %	16.52	5.93	19.25	4.00	6.244	0.06	0.85	0.72
Zn, %	13.42	-3.83	18.45	5.55	5.539	0.02	0.22	0.70
Apparent absorption								
Cu, %	25.07	0.20	25.50	15.05	4.792	<0.01	0.14	0.16
Mn, %	16.58	6.03	19.30	4.14	6.237	0.06	0.94	0.72
Zn, %	13.77	-3.42	18.70	5.74	5.544	0.02	0.23	0.71

[1]Treatment
[2]Period
[3]Treatment by period

CHAPTER 4.

SUPPLEMENTAL VITAMIN C IMPROVES MARBLING IN FEEDLOT CATTLE CONSUMING HIGH SULFUR DIETS

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ABSTRACT: The objective of this study was to examine the effects of supplemental rumen-protected vitamin C (**VC**) on live and carcass-based performance and antioxidant capacity of cattle consuming varying concentrations of dietary S. Angus-cross steers (n = 120) were blocked by initial BW (341 ± 11 kg) and assigned equally to one of six treatments evaluating three concentrations of dietary S (0.22, 0.34, and 0.55%, for low S (**LS**), medium S (**MS**), and high S (**HS**), respectively) and two concentrations of supplemental VC (0 or 10 $g \cdot h^{-1} \cdot d^{-1}$). Steers receiving VC supplemented diets consumed an average of 10.3 g of supplemental $VC \cdot h^{-1} \cdot d^{-1}$, and increasing dietary S linearly increased ($P < 0.01$) g of S consumed. Increasing dietary S decreased ($P < 0.01$) DMI, final BW, and ADG, and linearly increased ($P < 0.05$) rumen hydrogen sulfide and blood sulfhemoglobin concentrations. The inclusion of VC, regardless of S treatment, tended to increase ($P = 0.08$) plasma VC concentrations, specifically within the medium and high S diets ($P = 0.04$). Plasma total antioxidant capacity (d 90) linearly decreased ($P = 0.003$) and total liver glutathione (**GSH**; d 143) tended to decrease ($P = 0.08$) due to increased S intake.

Within the high S treatment, addition of VC decreased ($P = 0.04$) the ratio of oxidized-to-reduced GSH compared to HS alone. Increased dietary S and VC decreased ($P < 0.05$) plasma Cu concentrations, while VC increased ($P = 0.01$) plasma Fe concentrations. Linear decreases ($P < 0.02$) in marbling score, back-fat thickness (**BF**), yield grade, and HCW were observed as dietary S increased; however, the addition of VC to the HS diet increased ($P < 0.01$) BF, marbling scores, and the percentage of cattle grading Choice compared to the HS without VC. In conclusion, supplementation of VC to cattle receiving the high S diet improved marbling scores, while the exact mechanism for this improvement is unknown it may be related to a greater circulating VC available for lipid metabolism in these cattle.

INTRODUCTION

Ethanol industry co-products, such as dried distillers grains with solubles (**DDGS**), have become a common addition to feedlot cattle diets. However, due to the ethanol production process DDGS often contain elevated concentrations of S, potentially limiting the inclusion rate in cattle diets (Kwiatkowski et al., 2006). High dietary S (>0.4%) has been documented to have a detrimental effect on DMI, ADG, and HCW of feedlot cattle (Gibson et al., 1988; Richter et al., 2012), and may lead to development of S-induced polioencephalomalacia (**PEM**).

Vitamin C (**VC**), or ascorbate, has many roles in cellular metabolism, but has specific roles in oxidation-reduction reactions, as an enzyme cofactor, and in the synthesis of collagen (Rebouche, 1991). Currently, the NRC (1996) does not specify a dietary requirement by cattle for VC, as cattle naturally synthesize ascorbate from glucose.

Limited research is available concerning the daily requirements of VC for feedlot cattle. A decrease in serum VC was observed throughout the finishing period of Japanese Black cattle (Takahashi et al., 1999) and the inclusion of a rumen-protected VC to the diet of Japanese Black steers (12 to 24 months of age) improved marbling scores (Yano, 2001). The addition of VC to cell culture media enhanced the differentiation of pre-adipocytes to adipocytes (Torii et al., 1998), a critical step in marbling development (Lee et al., 2000). In addition to roles in marbling development, VC aids in the regeneration of reduced glutathione, an important antioxidant involved in the clearance of S metabolites from the body (Johnston et al., 1993; Truong et al., 2006). We hypothesized that VC supplementation to cattle consuming varying concentrations of dietary S would increase the antioxidant capacity and marbling potential of these cattle. This was evaluated by examining the effects of a rumen-protected VC on live and carcass-based performance and antioxidant capacity of cattle consuming varying concentrations of dietary S.

MATERIALS and METHODS

Procedures and protocols for the cattle experiment were approved by the Iowa State University Institutional Animal Care and Use Committee (protocol number 7-10-6986-B).

Animals and Experimental Design

Angus-cross calf-fed steers (n = 120) were purchased at a commercial auction barn and transported in August 2010, to the Iowa State University Beef Nutrition Farm (Ames, IA). Upon arrival, steers were weighed, de-wormed with Ivomec® Eprinex® Pour-On for Beef and Dairy (Merial Animal Health, Duluth, GA), vaccinated with Bovi-Shield® GOLD

FP 5L5 (Zoetis, New York City, NY) and One Shot Ultra®7 (Zoetis), and were identified with an ear tag. Steers were initially started on a common receiving diet for 28 d, followed by a series of 3 step-up diets in preparation for the finishing diet (Table 1).

At the initiation of the study (October, 2010), consecutive d weights were taken (prior to limit feeding) and steers were blocked by initial BW (341 ± 11 kg) and randomly assigned to pens of 4 steers within a block (5 pens per treatment). Cattle were assigned equally to 1 of 6 treatments evaluating 3 concentrations of dietary S (0.22, 0.34, and 0.55%, for low S (**LS**), medium S (**MS**), and high S (**HS**), respectively) and 2 concentrations of supplemental VC (0 or 10 $g \cdot h^{-1} \cdot d^{-1}$). Individual treatments consisted of: 1) low S (0.22% S; **LS**), corn-based diet, 2) LS + VC, 3) medium S (0.34% S; **MS**), 40% DDGS diet, 4) MS + VC, 5) high S (0.55% S; **HS**), MS + sodium sulfate, and 6) HS + VC. In this report, treatments will be referred to as the following: LS, LS + VC, MS, MS + VC, HS, and HS +VC, and throughout the paper, the combination of un-supplemented and VC supplemented treatments within a specific concentration of S will be referred to as the unabbreviated low S, medium S, and high S. Prior to receiving the assigned study diets, steers were implanted with Component E-S with Tylan (VetLife, Ivy Animal Health, Inc., Overland Park, KS), and 65 d later were implanted with Component TE-IS with Tylan (VetLife).

At the start of the experimental feeding period the finishing diet was limit fed at 1.5% BW and increased 0.25% each day for the first 5 d of the study, to decrease risk for acidosis and PEM. Consumption of the rumen-protected VC, containing 50% ascorbate (VitaShure C50; Balchem Corp., New Hampton, NY), was targeted at 10 g $VC \cdot h^{-1} \cdot d^{-1}$ (20 g of Vitasure C50); adjustments in VC inclusion were based on DM intake. Sodium sulfate

was included in the HS diet at 1.11% (DM basis) to provide an additional 0.21% S above the S concentration of the MS diets. Throughout the remainder of the study, interim weights were collected every 28 d, and consecutive final body weights were determined prior to harvest.

One steer from the HS treatment developed PEM symptoms on d 69 of the study, and was removed from the experiment and treated with dexamethasone and thiamin until fully recovered (data not included in analysis).

Sample Collection and Analytical Procedures

Feed offered to each pen and bunk scores were recorded daily and DMI was calculated. Samples of individual ingredients and total mixed rations (**TMR**) were collected weekly, and orts for each pen were collected monthly for DM determination. Samples were dried in a forced air oven at 70°C for 48 h. Feed efficiency (**G:F**) was determined every 28 d from the gain and DMI within that period. Sulfur analysis of weekly TMR samples and monthly pen orts was conducted according to the method described by Richter et al. (2012) and dietary S intake was calculated.

Jugular blood, approximately 25 mL, was collected into heparinized tubes (sodium heparin, Becton, Dickinson and Co., Franklin Lakes, NJ) for plasma and whole blood analysis from 2 steers per pen on d 0, 14, 28, 90, and 143. Plasma for trace mineral analysis was collected from the same 2 steers per pen using potassium EDTA tubes (Becton, Dickinson and Co.) on d 143. Blood was transported to the laboratory on ice and centrifuged at 1,000 × *g* for 10 min at 4°C. Plasma was aliquoted and stored at -80°C until the analysis of total antioxidant (**TA**) activity (Cayman Chemical Company, Ann Arbor,

MI; catalog #709001) and plasma trace mineral concentrations were determined. Plasma for mineral analysis was prepared with a 1:7 (v/v) dilution of plasma in 5% trace metal grade nitric acid. Samples were vortexed and centrifuged at 480 × *g* for 10 min at 4°C. Supernatant was collected and analyzed for mineral content using inductively coupled plasma optical emission spectrometry (Optima 7000 DV, Perkin Elmer, Waltham, MA). Standards consisting of National Institute of Standards and Technology bovine liver (Gaithersburg, MA) were included on each session to verify instrument accuracy. Plasma ascorbate (vitamin C) was assayed using a commercially available kit from Cayman Chemical Company (catalog #700420). Plasma was initially prepared with a combination of methanol, ultra-pure water, and diethylenetriaminepentaacetic acid at a ratio of 90:7.5:2.5. Samples were incubated on ice for 10 min and then centrifuged at 12,000 × *g* for 10 min at 4°C. The collected supernatant was stored at -80°C until further analysis, which was completed within 30 d of initial collection.

Rumen hydrogen sulfide (**H_2S**) and whole blood sulfhemoglobin concentrations were determined using the methods described by Drewnoski et al. (2012) at 6 h post feeding from one steer per pen on d 0, 14, 28, 90, and 143. Sulfhemoglobin concentrations were determined within 24 h of sample collection.

Liver was collected for analysis of glutathione (**GSH**) activity and mineral concentration via the biopsy technique described by Engle and Spears (2000) from 1 steer per pen on d 0 and 143. Biopsy samples were snap frozen in liquid nitrogen and transported back to the lab, where samples were stored at -80°C until further analysis. Glutathione activity was determined from samples collected on d 143 using the GSH assay kit (Sigma-Aldrich, St. Louis, MO; catalog # CS0260). Liver samples were dried in a

forced air oven, and approximately 0.25 g of dried liver was digested in 5 mL of trace metal grade nitric acid through closed vessel digestion in a Mars Xpress microwave (CEM Corporation, Matthews, NC), and brought to final volume of 25 mL with deionized water. Liver mineral analysis was conducted as previously described for plasma mineral analysis, and final mineral concentrations were adjusted to a per gram of tissue.

Ultrasound measures of all steers were conducted by a certified technician on d 0 and 90 for rib-eye area (**REA**), percent intramuscular fat of the REA, back-fat thickness (**BF**), and rump fat thickness. Steers were harvested on d 149 when greater than 60% of steers in a pen were estimated by visual appraisal to have at least 1.27 cm of back-fat. Steers were harvested at a commercial packing facility in Denison, IA where individual identification was maintained with each carcass following harvest. Carcasses were chilled for 24 h, after which carcasses were ribbed between the 12^{th} and 13^{th} rib and graded according to USDA standards. Carcass data were collected at the plant by representatives of Tri-County Carcass Futurity (Iowa State University Beef Extension, Lewis, IA) who were masked to treatments. Data from 3 steers were not collected because carcasses were railed out at the packing plant. Data collected from harvested steers (n = 116) included HCW, marbling score, BF, KPH, REA, quality grade (**QG**) and yield grade (**YG**). The carcass-adjusted performance data calculation of final BW was determined by dividing HCW by the average dressing percentage of 64%. As a means to account for gut fill, a 4% pencil shrink was applied to all live BW measures to calculate final ADG.

Statistical Analysis

Data were analyzed by ANOVA as a randomized complete block design using the Mixed Procedure of SAS (SAS Institute Inc., Cary, NC). The model for the analysis of performance, ultrasound measures, TA capacity, GSH, plasma and liver mineral analysis, and carcass data included the fixed effect of treatment, while block was the random effect. The GenMod procedure of SAS was used to determine differences in the percentages of QG and YG within treatments. Dietary S intake, DMI, ADG, G:F, VC intake, rumen H_2S, blood sulfhemoglobin, and plasma ascorbate data were analyzed as repeated measures and included the fixed effects of treatment, time of sampling, and the interaction. Block was the random effect and time was the repeated effect. Based on the Akaike Information Corrected Criterion, unstructured was selected as the covariance structure for repeated measures analysis of rumen H_2S, while variance components was used in analysis of all other repeated measures. Pen was used as the experimental unit for all data analysis (n = 5 per treatment). Day 0 values were used in a covariate analysis in all analyses except plasma and liver mineral and GSH data, as these data were only collected on d 143. Initial BW was used as a covariate for final BW and HCW data. The Cook's D outlier test of SAS was used to identify outliers. Three single df contrast statements were constructed prior to analysis: A) VC versus no VC, B) linear effect of S, and C) VC within high S (0.55% S). Significance was declared at $P \leq 0.05$ and tendencies were declared from $P = 0.06$ to 0.10. The values reported in the tables are least squares means.

RESULTS

Intake, growth, and performance

Steer performance results are presented in Figure 1 and Table 2. Based on repeated measures analysis DMI and S intake demonstrated a treatment by month interaction ($P < 0.04$). The DMI treatment by month interaction appears to be the result of the differential DMI within the medium S treatments. From d 38 to 65 the MS treatment was consuming approximately 0.81 kg per d more ($P = 0.02$) than the MS + VC cattle, and MS cattle tended ($P = 0.06$) to consume approximately 0.76 kg per d more during d 94 to 121 than the MS + VC treatment. This trend is reflected in the S intake, which by design, increased ($P < 0.001$) with greater inclusion of dietary S. The treatment by month interaction ($P < 0.001$) of S intake appears to be due to greater S intakes by the MS cattle during d 38 to 65 ($P = 0.005$) and 94 to 121 ($P = 0.10$) compared with the MS + VC cattle. Additionally, the HS + VC cattle had a greater S intake, compared to the HS treatment, during d 94 to 121 ($P = 0.09$) and d 122 to 149 ($P = 0.02$) which may be a due to the slightly greater ($P = 0.09$) S concentration in the HS + VC diet based on TMR samples collected throughout the trial.

Repeated measures analysis revealed an ADG treatment by month interaction ($P < 0.001$) which may be the result of an increase ($P < 0.001$) in ADG by the LS + VC and HS + VC during d 66 to 93. Additionally, during d 122 to 149, the ADG of the MS cattle tended to be greater ($P = 0.07$) than the MS + VC cattle, while the ADG of the MS + VC cattle were comparable ($P = 0.79$) to those of the high S treatments. The treatment by month interaction ($P < 0.001$) for G:F is primarily attributed to the increase ($P = 0.05$) in G:F by the HS + VC treatment from d 38 to 65 to d 66 to 93, while the MS treatment

decreased to values below the MS + VC ($P = 0.07$) and HS + VC ($P = 0.03$) during d 66 to 93. Dry matter intake, final BW, and ADG linearly decreased ($P < 0.001$) as dietary S increased. The greatest impact of dietary S was observed within the high S (0.55% S) treatments, and these treatments are the driving force for the linear effects of S on DMI ($P < 0.001$), final BW ($P = 0.001$), and ADG ($P = 0.001$). No difference ($P = 0.31$) was noted in VC consumption among VC supplemented cattle, as steers averaged 10.3 $g \cdot h^{-1} \cdot d^{-1}$ across the study (10.61 g, 10.37 g, and 9.88 g $VC \cdot h^{-1} \cdot d^{-1}$, LS + VC, MS + VC, and HS + VC respectively). No effect of VC supplementation ($P \geq 0.89$) was observed on live performance measures.

Ultrasound measures are presented in Table 3. On d 90, rump fat, BF, and percent intramuscular fat were all linearly decreased ($P < 0.03$) by increasing dietary S. Interestingly, the addition of VC to any of the 3 concentrations of dietary S tended to increase ($P = 0.08$) BF values; however, only in the HS + VC treatment was an increase ($P = 0.05$) in intramuscular fat observed relative to the HS cattle.

Indicators of S exposure

Rumen H_2S concentrations are presented in Figure 2. Based on repeated measures analysis, H_2S concentrations demonstrated a treatment by day interaction ($P = 0.001$). The treatment by day interaction is primarily being driven by the high S treatments, and may be attributed to the slightly greater S intake ($P = 0.09$) by the HS + VC cattle. The increased inclusion rate of dietary S resulted in a linear increase ($P < 0.001$) in H_2S concentrations. Hydrogen sulfide values peaked at 0.48 g/m^3 (in the HS cattle) on d 14. Because the VC

product should be mostly rumen undegradable it was anticipated that there would be no effect of VC on H_2S concentrations and none was noted ($P = 0.46$).

Sulfhemoglobin concentrations are presented in Table 4. Based on repeated measures analysis, sulfhemoglobin, total hemoglobin, and percent sulfhemoglobin concentrations demonstrated a treatment by day interaction ($P \leq 0.03$). The treatment by day interaction of sulfhemoglobin concentrations and the percent sulfhemoglobin is primarily being driven by the peak sulfhemoglobin values at d 28, especially within the medium S treatments. Interestingly, sulfhemoglobin concentrations were greater ($P = 0.02$) in cattle receiving VC, regardless of S inclusion. This effect is mostly caused by the differences among the HS and HS + VC cattle, and may again be related to the tendency for increased ($P = 0.09$) consumption of S by the HS + VC cattle.

Antioxidant measures

Plasma ascorbate concentrations are presented in Figure 3. The inclusion of VC to all concentrations of dietary S tended to increase ($P = 0.08$) plasma ascorbate concentrations, with the most notable increase being within the high S treatment ($P = 0.02$). A treatment by day interaction ($P = 0.03$) of plasma ascorbate was observed, specifically noted within the high S steers. Steers consuming the high S diet demonstrated a decrease ($P = 0.005$) in plasma ascorbate between d 28 and 90, but during the final 53 d of the study recovered ($P = 0.03$) plasma ascorbate concentrations to similar values as the other five treatments. The supplementation of the high S cattle with VC prevented the decrease ($P = 0.005$) in ascorbate between d 28 and 90.

The TA capacity of cattle was linearly decreased ($P = 0.003$; data not shown) as dietary S increased on d 90, and tending ($P = 0.10$) to follow the same trend on d 143. The decrease in TA capacity due to S seems to be primarily driven by the HS treatment. Vitamin C supplementation did not influence TA capacity on either d 90 ($P = 0.17$) or d 143 ($P = 0.92$).

Liver GSH concentrations are presented in Table 5. Total liver GSH tended to decrease ($P = 0.08$) in cattle as dietary S increased. Vitamin C supplementation did not influence ($P = 0.71$) available reduced GSH. No influence of dietary S concentration ($P = 0.13$) or addition of VC ($P = 0.38$) was observed in oxidized GSH concentrations. The ratio of oxidized-to-reduced GSH was greater ($P = 0.04$) in the liver of steers fed HS without VC (0.28 ± 0.15) compared to HS + VC steers (0.07 ± 0.15).

Plasma and liver trace minerals

Plasma and liver trace mineral concentrations are presented in Table 6. Increasing dietary S linearly decreased ($P = 0.003$) plasma Cu concentrations in steers on d 143. The addition of VC to any concentration of S also decreased ($P \leq 0.05$) plasma and liver Cu concentrations compared to those steers not receiving VC, while plasma Fe concentrations were increased ($P = 0.01$) in VC supplemented steers compared with steers not receiving supplemental VC. Liver Fe concentrations were not affected ($P = 0.53$) by supplemental VC. Liver Zn concentrations were decreased ($P = 0.007$) due to supplemental VC and tended to linearly decrease ($P = 0.07$) with a greater dietary S inclusion.

Carcass characteristics

Carcass characteristics and the distribution of QG and YG are presented in Tables 7 and 8, respectively. Linear decreases ($P < 0.02$) in HCW, YG, marbling score, and BF were observed as diets increased in dietary S concentration. Again, the high S diets are the primary driving force behind the linear effects of increasing S on carcass traits. Interestingly, the inclusion of VC to the HS treatment eliminated some of the depressive effects of HS on carcass traits, as the inclusion of VC increased ($P = 0.002$) marbling scores from 398 to 470 (± 13.4; 300 = slight, 400 = small; 500 = modest) in the HS and HS + VC treatments, respectively, and increased ($P = 0.01$) BF scores from 1.0 to 1.24 (± 0.06) cm in the HS and HS + VC, respectively. However, the addition of VC to the LS diet resulted in decreased ($P = 0.004$) marbling scores (443 ± 13.4) compared to steers receiving LS (511 ± 13.4). The inclusion of VC to the HS diet resulted in a greater ($P = 0.01$) percentage of cattle that graded Choice and a decreased ($P = 0.02$) percentage grading Select compared to the HS treatment. Addition of VC to any concentration of dietary S resulted in a greater ($P = 0.001$) percentage of KPH fat.

DISCUSSION

Our objective was to examine the effects of supplemental rumen protected VC on live and carcass based performance and antioxidant capacity of cattle consuming a low, medium, or high S diet. Previous research indicates that feeding high S corn co-products decreases live cattle performance, resulting in lighter HCW and a decreased QG, thus impacting carcass value (Gibson et al., 1988; Zinn et al., 1997; Richter et al., 2012). Cattle in the present study experienced a similar loss of performance, as decreases in HCW, BF,

marbling score, and YG were observed in cattle consuming the HS diet. Interestingly, while the least amount of marbling was observed in the HS treatment, the addition of VC to the HS diet recovered marbling scores to values comparable with the low S and medium S treatments, even though the HS + VC cattle had lighter HCW.

The use of ultrasound, conducted on d 0 and 90, enabled the authors to monitor the nutritional impact of dietary S and VC on body composition over this time period. Initial ultrasound measures indicated the cattle assigned to HS + VC treatment had a smaller REA, which was reflected in the final carcass measures. The decrease in rump fat, BF, and intramuscular fat values on d 90 suggest high dietary S may be detrimental to lipid accumulation and thereby increase the risk for decreased QG of beef carcasses at harvest. Additionally, the depression in DMI, due to high dietary S, may be a contributing factor to the depression in carcass traits on d 90 of the HS treatment. Interestingly, the addition of VC to diets increased lipid accumulation on d 90; specifically BF (regardless of dietary S) and intramuscular fat in HS + VC treatment was greater.

Similar improvements in marbling score due to VC supplementation were observed by Ohashi et al. (2000) and Yano (2001), where the addition of a rumen-protected VC source during the middle finishing period (12 to 24 months of age) increased marbling scores of Japanese Black long-fed steers. The results of these studies prompted the addition of the low S corn-based diet to the current study, with the hypothesis that the high starch content of the diet would maximize the marbling opportunity in these cattle. However, the addition of VC to the LS diet decreased marbling scores compared to the LS without VC diet. It is unclear why LS + VC cattle demonstrated greater intramuscular fat on d 90 via ultrasound detection and yet had decreased marbling scores at harvest on d 143. This effect

may be attributed to competition for a common transporter between glucose and VC, as glucose and the oxidized form of VC, dehydroascorbate, are both recognized by the glucose transporter family (Kodaman and Behrman, 1999).

The present study utilized $NaSO_4$ to provide additional S in the high S treatments. Increasing the availability of Na may have influenced the absorption of glucose via sodium-glucose transporter-1 from the small intestine because of its dependence on a Na electrochemical gradient. Manipulating glucose absorption, through increased availability of Na for transport, may be contributing to the increase in intramuscular lipid accumulation observed in HS + VC cattle as Smith and Crouse (1984) noted glucose is the preferred substrate for intramuscular adipose tissue deposition. The authors are unaware of any literature concerning intestinal transport mechanisms for VC in cattle, as cattle are able to naturally synthesize VC and therefore any intestinal absorption is believed to occur via passive diffusion rather than via an active transport mechanism such as sodium-glucose transporter-1 (Combs, 2008).

While the mechanism of action by which VC may be driving an increase in marbling, BF, and KPH is unclear, potential mechanisms may include: a general increase in glucose uptake by adipocytes via decreased oxidative stress (Paolisso et al., 1994), an alteration of the extracellular matrix (Nakajima et al., 1998; Hausman et al., 2009) which may be related to the role of VC in collagen production and the importance of preadipocyte adherence to the surrounding extracellular matrix (specifically collagen and fibronectin) during differentiation, or a greater glycerol-3 phosphate dehydrogenase (**GPDH**) activity (Ono et al., 1990; Kawada et al., 1990; Torii et al., 1998; Lee et al., 2000).

Glycerol-3 phosphate dehydrogenase is an essential component for lipid biosynthesis, by converting glycerol-3 phosphate to glycerol for incorporation into triglycerides. Previous research indicates the addition of L-ascorbic acid and L-ascorbic acid 2-phosphate to cultured bovine adipocytes and the 3T3-L1 cell line increases GPDH activity and subsequent lipid accumulation (Ono et al., 1990; Kawada et al., 1990; Torii et al., 1998; Lee et al., 2000). Interestingly, the cells experiencing the greatest increase in GPDH activity were those harvested from subcutaneous or peri-renal adipose tissue compared to intramuscular adipose tissue (Smith and Crouse, 1984; Lee et al., 2000; Bonnet et al., 2007); these data may provide an explanation as to the increase in KPH experienced in the present study by cattle consuming VC. Additional research is required to identify the exact mechanism of action by which VC may be increasing the fatness of these cattle.

Hydrogen sulfide production due to metabolism by sulfate-reducing bacteria in the rumen is believed to be the mechanism by which high sulfate diets can be toxic to ruminants. The high S steers in the present study experienced peak H_2S values of 0.48 g/m^3 on d 14, which is consistent with the observations of Loneragan et al. (1997) and Richter et al. (2012) who noted the peak in H_2S production occurred within the first 15 to 35 d on a high S diet, and during this time cattle are more susceptible to the development of PEM.

Sulfhemoglobin is formed when S in the blood irreversibly binds to the Fe molecule in hemoglobin taking the place of oxygen, thereby decreasing the oxygen carrying capacity of the blood (Keilin, 1933). In the present study, sulfhemoglobin values increased in response to greater concentrations of dietary S and also the inclusion of VC. It is not entirely clear why VC inclusion resulted in an increase in the sulfhemoglobin

concentrations in these cattle, as H_2S concentrations did not differ due to VC inclusion and in previous studies sulfhemoglobin values were correlated with H_2S values (Drewnoski et al., 2012). Similar to H_2S production, the increase in sulfhemoglobin in the HS + VC treatment may be related to the greater S intake by these steers. Interestingly, it was observed by Nichol et al. (1968) that sulfhemoglobin could be produced through the reaction of hemoglobin with ascorbate and H_2S in the presence of phenylhydroxylamine, suggesting ascorbate was providing reducing power. Therefore, the supplementation of VC to the diet containing any concentration of dietary S may have provided enough additional reducing power to encourage the formation of sulfhemoglobin. It is important to note that while sulfhemoglobin (as a percent of total hemoglobin) increased as the dietary S increased, these cattle were well below the toxicity threshold of 1% sulfhemoglobin (of total hemoglobin; Triapirux et al., 2008).

While limited research data are available concerning plasma ascorbate concentrations of feedlot cattle, existing data indicate great variability among plasma ascorbate concentrations in cattle across all aspects of production. In a review, Smith et al. (2009) reported plasma ascorbate concentrations of healthy beef cattle ranged from 2.4 to 4.7 mg/L; however, cattle in the present study demonstrated plasma ascorbate values of 0.3 to 0.7 mg/L across the finishing period. These values are much less than values previously reported, which may be related to differences in detection methods utilized: in the present study florescence spectrometry was used compared to methods used by others, including a photoelectric colorimeter assay (Mindlin and Butler, 1938) or an HPLC method (Haiying et al., 2003).

The decrease in plasma ascorbate of the HS cattle between d 28 and 90 suggests the natural production of VC may not be sufficient to meet the steer's needs while consuming a high S diet. Takahashi et al. (1999) observed a similar decline in ascorbate was observed across the finishing period when a rumen-protected source of VC was included in the diet; however, those cattle were not consuming a high S diet. Further research is warranted to determine if high S diets without VC supplementation do indeed result in a drop in plasma ascorbate. In the present study, plasma ascorbate concentrations of the HS cattle began to recover to comparable values with the LS and MS treatments between d 90 and 143 which may represent a reallocation of glucose toward greater VC production in the liver. A greater use of glucose for VC production may help explain the decrease in marbling scores of the HS cattle, as glucose is the preferred substrate for intramuscular adipose tissue development (Smith and Crouse, 1984). Results of the present study in combination with those of Takahashi et al. (1999) suggest the inclusion of a rumen-protected VC source may be beneficial to maintain circulating ascorbate concentrations across the finishing period. Because VC is synthesized in the liver of cattle from glucose, supplemented VC may provide a sparing mechanism allowing more glucose to be available for marbling development.

Similar to plasma ascorbate, limited research data are available concerning plasma TA capacity in cattle. The data available suggests a decrease in serum TA capacity of transport-stressed beef calves (Chirase et al., 2004); alternately, Castillo et al. (2006) did not notice a difference in serum TA capacity of dairy cows during lactation. Results of the present study indicate dietary S decreases plasma TA capacity over the finishing period. This decrease suggests cattle receiving a high S diet may experience more oxidative stress,

leading to a depressed TA capacity. It is unclear why the addition of VC to the MS and HS treatment decreased the TA capacity on d 90, as VC should be contributing to the TA capacity. However, as a water soluble vitamin, VC has limited storage capacity within the body and therefore may not have been directly affecting the circulating TA capacity. Another explanation may be related to the increase in plasma Fe concentration in cattle supplemented with VC, as a greater amount of Fe may be contributing to oxidative conditions resulting in a depletion of TA capacity.

Glutathione is also an essential antioxidant in the body, aiding in the removal of reactive oxygen species and free radicals (Pastore et al., 2003). Truong et al. (2006) observed the depletion of GSH by H_2S in cell culture, suggesting that GSH plays a vital role in the detoxification of H_2S due to excess S. Because VC is able to donate electrons to regenerate reduced GSH, it was hypothesized that the inclusion of VC would increase the availability of reduced GSH. Only the LS + VC treatment tended to have a greater concentration of reduced GSH in support of our hypothesis; the MS + VC and HS + VC treatments did not experience an increase the availability of reduced GSH. It appears the elevated S load may be responsible for the depletion of GSH concentrations in the HS cattle and the addition of VC was not sufficient to overcome this stress.

In healthy cells, oxidized GSH should comprise no more than approximately 10% of the total GSH, and percentages exceeding this are indicative of oxidative stress (Ithayaraja, 2011). Within the high S treatments, the ratio of oxidized-to-reduced GSH was greater in liver from steers fed HS without VC (0.28 ± 0.15) compared to HS + VC steers (0.09 ± 0.15), indicating the HS without VC cattle were experiencing some oxidative stress as 28% of the total GSH was in the oxidized form.

These data support the idea that high S diets contribute to a decreased TA capacity in cattle. Because dietary S is removed from the body via GSH, the consumption of high S may limit the availability of reduced GSH for other critical antioxidant functions in the body. Furthermore, high dietary S can impact the availability of other body antioxidants such as superoxide dismutase by decreasing the availability of the critical trace minerals necessary for optimal function. While superoxide dismutase activity was not determined in the present study, the increase in oxidized-to-reduced GSH ratio in HS treatment may be related to an increased reliance on GSH to remove both free radicals and S.

Sulfur and VC are just two of the many factors that can influence the absorption of trace minerals, and due to the presence of both in the present study the analysis of trace mineral status was essential. Cattle in the present study experienced a decrease in plasma Cu concentration as dietary S was increased, which may be attributed to an antagonistic interaction between Cu and S (Davis and Mertz, 1987; Phillippo et al., 1987). In the rumen, dietary S and Mo can bind Cu to form insoluble and biologically unavailable complexes called thiomolybdates, limiting the availability of Cu to cattle (Phillippo et al., 1987). Interestingly, the addition of VC to any concentration of S decreased the plasma and liver Cu concentrations, while plasma Fe concentrations were increased. Because cattle are able to naturally synthesize VC there has been little concern with supplemental VC and its influence on the mineral status of cattle; however, it has been documented in rats, guinea pigs, and humans that VC negatively impacts Cu status by decreasing absorption (Van den Berg et al., 1994; Milne and Omaye, 1980; Milne et al., 1988). Vitamin C aids in the reduction of Fe from the ferric to ferrous state, which increases absorption across the small intestine (Milne and Omaye, 1980; Van den Berg and Beynen, 1992).

In conclusion, supplementation of VC to cattle consuming high S diets improved marbling scores, while VC inclusion to the low and medium S treatments had limited benefits. Although the exact mechanism for the improvement in carcass quality in the HS + VC steers is unknown, it may be related to the greater circulating ascorbate found in the HS + VC cattle compared to the HS cattle. Because TA capacity was decreased by increasing the concentration of dietary S it is possible that plasma ascorbate was being used in these steers in place of other functional antioxidants. This may be why plasma ascorbate decreased in steers receiving the HS diet. The addition of VC to the HS diet prevented the decrease in plasma ascorbate concentrations during the initial 90 d of the study, meaning these cattle had more ascorbate available for functions related to lipid metabolism, perhaps explaining the observed improved marbling scores in these cattle.

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Table 1. Ingredient composition and chemical analysis of finishing diets (% DM basis)

Item	Low S[1]	Medium S[1]	High S[1,6]
Corn	69.7	48.0	48.0
Corn dried distillers grains[2]	18.0	40.0	38.9
Chopped hay	9.0	9.0	9.0
Limestone	2.0	2.0	2.0
Salt	0.31	0.31	0.31
Vitamin A premix[3]	0.10	0.10	0.10
Trace mineral premix[4]	0.035	0.035	0.035
Rumensin90[5]	0.016	0.016	0.016
Sodium sulfate[6]	--	--	1.11
Urea	0.80	--	--
Analyzed composition			
CP, %	14.3	16.6	16.6
NEg, Mcal/kg DM	1.3	1.3	1.3
S[7], %	0.22	0.34	0.55

[1]Vitashure C, provided by Balchem Corp., replaced DDGS at 0.215% DM to achieve 10 g of VC per steer per day; vitamin C intake averaged 10.3 g per steer per day

[2]Five loads of DDGS from Lincoln Way Energy (Nevada, IA) were used during the trial with S concentrations of 0.72%, 0.67%, 0.70%, 0.79%, and 0.55%

[3]Vitamin A premix contained 4,400,000 IU/kg

[4]Provided per kg of diet: 30 mg Zn as $ZnSO_4$; 20 mg Mn as $MnSO_4$; 0.5 mg I as $Ca(IO_3)_2(H_2O)$; 0.1 mg Se as Na_2SeO_3; 10 mg Cu as $CuSO_4$; and 0.1 mg Co as $CoCO_3$

[5]Provided at 27 g/t diet (donated by Elanco Animal Health)

[6]Sodium sulfate added to the diet, at the expense of DDGS, to increase the percent S of the medium S diet by 0.36% S

[7]Percent S for low, medium, and high diets are based on repeated measures analysis of samples collected over the 149 d study

Table 2. Effect of supplemental vitamin C (10 g vitamin C·steer^{-1}·d^{-1}) on gain and efficiency of steers fed a low S (0.22%), medium S (0.34%), or high S (0.55%) diet for 143 days

Diet sulfur	Low[1]		Medium[1]		High[1]			Contrast Statement[2]		
Diet vitamin C	-	+	-	+	-	+	SEM	A	B	C
Pens/treatment (n)	5	5	5	5	5	5				
Initial BW, kg	342	342	341	343	340	340	10.6	0.05	0.01	0.79
Live performance[3]										
Final BW, kg	575	572	576	572	543	553	6.78	0.91	0.001	0.32
ADG, kg/d[4]	1.65	1.66	1.69	1.61	1.43	1.48	0.04	0.95	0.001	0.31
d 1-37	2.24	2.21	2.41	2.16	1.90	1.92	0.08	0.12	0.001	0.13
d 38-65	1.58	1.51	1.87	1.76	1.39	1.38	0.13	0.87	0.03	0.83
d 66-93	1.62	1.74	1.56	1.75	1.55	1.71	0.12	0.002	0.04	0.08
d 94-121	1.41	1.49	1.33	1.28	1.21	1.26	0.10	0.62	0.32	0.65
d 122-149	1.37	1.38	1.30	1.14	1.12	1.15	0.07	0.31	0.002	0.55
G:F[5]	0.16	0.16	0.16	0.16	0.15	0.15	0.004	0.89	0.03	0.32
d 1-37	0.24	0.24	0.25	0.23	0.23	0.24	0.007	0.32	0.32	0.66
d 38-65	0.15	0.14	0.16	0.16	0.13	0.13	0.013	0.80	0.14	0.77
d 66-93	0.16	0.15	0.14	0.17	0.16	0.17	0.009	0.002	0.47	0.03
d 94-121	0.13	0.14	0.12	0.12	0.13	0.13	0.009	0.88	0.97	1.0
d 122-149	0.14	0.14	0.13	0.12	0.13	0.13	0.007	0.17	0.002	0.72

Carcass-adjusted performance[6]										
Final BW, kg	581	580	589	584	549	559	7.5	0.83	0.002	
ADG, kg/d	1.62	1.58	1.67	1.60	1.43	1.44	0.04	0.31	0.001	
G:F	0.16	0.15	0.17	0.16	0.16	0.16	0.004	0.43	0.83	0.60

[1]Treatments: Low (-): low sulfur; Low (+): low sulfur + 10 g·steer^{-1}·d^{-1} vitamin C; Medium (-): medium sulfur; Medium (+): medium sulfur + 10 g·steer^{-1}·d^{-1} vitamin C; High (-): high sulfur; High (+): high sulfur + 10 g·steer^{-1}·d^{-1} vitamin C

[2]Contrast statements: A= vitamin C versus no vitamin C; B = linear effect of sulfur; C = vitamin C within high sulfur

[3]Live performance values based on measured live BW with a 4% pencil shrink applied

[4]Treatment by month ($P < 0.001$)

[5]Treatment by month ($P < 0.001$)

[6]Carcass adjusted performance values based on final BW calculated from HCW divided by the average dressing percent of 64% for all treatments; ADG and G:F were calculated over the total 149 d study

Table 3. Effect of supplemental vitamin C (10 g·steer^{-1}·d^{-1} vitamin C; VC) on ultrasound carcass characteristics of cattle fed a low S (0.22%), medium S (0.34%), or high S (0.55%) diet for 149 days

Diet sulfur	Low[1]		Medium[1]		High[1]			Contrast Statement[2]		
Diet vitamin C	-	+	-	+	-	+	SEM	A	B	C
Pens/treatment (n)	5	5	5	5	5	5				
Initial, d 0										
Rump fat, cm	0.50	0.53	0.52	0.52	0.50	0.47	0.05	0.86	0.39	0.78
Back-fat, cm	0.41	0.44	0.46	0.43	0.40	0.41	0.02	0.81	0.22	0.86
REA, cm^2	59.8	62.1	59.1	61.5	62.0	56.9	2.0	0.91	0.29	0.01
REA percent fat	2.97	3.16	3.04	3.02	2.92	3.13	0.11	0.16	0.72	0.16
Mid-point, d 90[3]										
Rump fat, cm	1.31	1.17	1.40	1.30	1.12	1.16	0.05	0.13	0.03	0.69
Back-fat, cm	1.14	1.19	1.21	1.21	1.00	1.12	0.03	0.08	0.006	0.02
REA, cm^2	81.7	81.9	81.1	82.0	79.6	80.8	1.2	0.95	0.59	0.66
REA percent fat	4.36	4.81	4.88	4.63	3.93	4.24	0.12	0.12	<0.001	0.05

[1]Treatments: Low (-): low S; Low (+): low S + 10 g·steer^{-1}·d^{-1} vitamin C; Medium (-): medium S; Medium (+): medium S + 10 g·steer^{-1}·d^{-1} vitamin C; High (-): high S; High (+): high S + 10 g·steer^{-1}·d^{-1} vitamin C

[2]Contrast statements: A= vitamin C versus no vitamin C; B = linear effect of sulfur; C = vitamin C within high sulfur

[3]Day 0 values used as a covariate; values reported are covariate adjusted means

Table 4. Effect of supplemental vitamin C (10 g·steer^{-1}·d^{-1} vitamin C; VC) on sulfhemoglobin concentrations of cattle fed a low S (0.22%), medium S (0.34%), or high S (0.55%) diet for 143 days

Diet sulfur	Low[1]		Medium[1]		High[1]			Contrast Statement[2]		
Diet vitamin C	-	+	-	+	-	+	SEM	A	B	C
Steers/treatment (n)	5	5	5	5	5	5				
Sulfhemoglobin, mg/d[3]	29.4	35.3	48.8	46.6	44.3	58.7	5.06	0.07	<0.001	0.02
d 14	17.9	37.1	41.4	44.7	48.1	61.7	5.97	0.01	0.002	0.16
d 28	40.6	48.9	74.5	61.4	59.3	75.7	6.14	0.97	<0.001	0.44
d 90	19.6	26.1	45.7	50.8	46.3	52.1	6.50	0.05	<0.001	0.30
d 143	39.7	29.1	33.6	29.6	23.4	45.6	9.32	0.30	0.72	0.04
Total hemoglobin, g/dL[3,4]	13.8	14.0	13.8	13.6	14.1	13.3	0.50	0.53	0.75	0.24
d 14	13.9	14.9	13.7	13.4	15.0	13.7	0.54	0.23	0.57	0.11
d 28	11.6	13.4	12.1	11.6	12.3	11.9	0.92	0.68	0.51	0.71
d 90	15.0	13.9	14.9	14.5	14.5	13.5	0.42	0.02	0.53	0.70
d 143	14.6	13.8	14.5	15.0	14.7	13.9	0.51	0.49	0.30	0.32
Sulfhemoglobin[3,4,5], %	0.23	0.28	0.38	0.37	0.34	0.45	0.05	0.25	0.01	0.13
d 14	0.15	0.31	0.31	0.35	0.36	0.46	0.05	0.02	0.02	0.02
d 28	0.42	0.33	0.65	0.61	0.54	0.65	0.09	0.18	0.005	0.16
d 90	0.11	0.22	0.29	0.33	0.32	0.38	0.05	0.03	0.001	0.22
d 143	0.24	0.24	0.26	0.19	0.15	0.32	0.06	0.31	0.61	0.06

[1]Treatments: Low (-): low S; Low (+): low S + 10 g·steer^{-1}·d^{-1} vitamin C; Medium (-): medium S; Medium (+): medium S + 10 g·steer^{-1}·d^{-1} vitamin C; High (-): high S; High (+): high S + 10 g·steer^{-1}·d^{-1} vitamin C

[2]Contrast statements: A= vitamin C versus no vitamin C; B = linear effect of sulfur; C = vitamin C within high sulfur

[3]Day 0 values used as a covariate

[4]Day ($P < 0.001$), and treatment × day ($P \leq 0.03$)

[5]Sulfhemoglobin as a percentage of total hemoglobin

Table 5. Effect of supplemental vitamin C (10 g·steer^{-1}·d^{-1} vitamin C; VC) on liver glutathione (reduced and oxidized) concentrations of cattle fed a low S (0.22%), medium S (0.34%), or high S (0.55%) diet for 143 days

Diet sulfur	Low[1]		Medium[1]		High[1]			Contrast Statement[2]		
Diet vitamin C	-	+	-	+	-	+	SEM	A	B	C
Steers/treatment (n)	5	5	5	5	5	5				
Glutathione, total (µM/g)	2.51	3.22	3.27	4.06	2.74	2.02	0.57	0.41	0.08	0.19
Glutathione, reduced (µM/g)	1.73	2.60	2.63	3.06	1.98	1.85	0.56	0.20	0.24	0.77
Glutathione, oxidized (µM/g)	0.28	0.31	0.32	0.28	0.26	0.09	0.15	0.38	0.13	0.17
Oxidized:Reduced	0.17	0.13	0.13	0.16	0.28	0.07	0.11	0.21	0.61	0.04

[1]Treatments: Low (-): low S; Low (+): low S + 10 g·steer^{-1}·d^{-1} vitamin C; Medium (-): medium S; Medium (+): medium S + 10 g·steer^{-1}·d^{-1} vitamin C; High (-): high S; High (+): high S + 10 g·steer^{-1}·d^{-1} vitamin C

[2]Contrast statements: A= vitamin C versus no vitamin C; B = linear effect of sulfur; C = vitamin C within high sulfur

Table 6. Effect of supplemental vitamin C (10 g·steer^{-1}·d^{-1} vitamin C; VC) on plasma and liver trace mineral concentrations of cattle fed a low S (0.22%), medium S (0.34%), or high S (0.55%) diet for 143 days

Diet sulfur	Low[1]		Medium[1]		High[1]			Contrast Statement[2]		
Diet vitamin C	-	+	-	+	-	+	SEM	A	B	C
Plasma mineral										
Steers/treatment (n)	5	5	5	5	5	5				
Cu, mg/L	1.10	0.95	0.93	0.84	0.86	0.81	0.06	0.05	0.003	0.53
Fe, mg/L	1.77	2.30	2.18	2.07	2.08	2.22	0.11	0.01	0.21	0.22
Mg, mg/L	20.7	22.3	21.2	22.8	21.7	20.2	0.64	0.27	0.29	0.10
Zn, mg/L	1.03	1.01	1.10	1.15	1.05	0.94	0.06	0.58	0.41	0.18
Liver mineral										
Steers/treatment (n)	5	5	5	4	5	4				
Cu, mg/kg	244	237	271	217	250	234	16.6	0.03	0.94	0.41
Fe, mg/kg	188	196	198	200	189	199	13.2	0.53	0.95	0.50
Mn, mg/kg	8.8	10.2	9.9	9.4	10.1	10.0	0.58	0.58	0.29	0.90
Zn, mg/kg	131	107	133	117	112	111	6.3	0.007	0.07	0.93

[1]Treatments: Low (-): low S; Low (+): low S + 10 g·steer^{-1}·d^{-1} vitamin C; Medium (-): medium S; Medium (+): medium S + 10 g·steer^{-1}·d^{-1} vitamin C; High (-): high S; High (+): high S + 10 g·steer^{-1}·d^{-1} vitamin C

[2]Contrast statements: A= vitamin C versus no vitamin C; B = linear effect of sulfur; C = vitamin C within high sulfur

Table 7. Effect of supplemental vitamin C (10 g·steer^{-1}·d^{-1} vitamin C; VC) on carcass characteristics of steers fed a low S (0.22%), medium S (0.34%), or high S (0.55%) diet for 149 days

Diet sulfur	Low[1]		Medium[1]		High[1]			Contrast Statement[2]		
Diet vitamin C	-	+	-	+	-	+	SEM	A	B	C
Pen per treatment (n)	5	5	5	5	5	5				
HCW, kg	369	365	371	367	350	356	8.29	0.85	0.007	0.40
Dressing %	64.0	64.1	64.8	65.0	64.3	64.3	0.002	0.59	0.71	0.90
Calculated YG	3.45	3.28	3.26	3.20	2.90	3.16	0.14	0.94	0.02	0.21
KPH, %	2.08	2.34	2.21	2.43	2.26	2.18	0.05	<0.001	0.77	0.06
QG[3]	3.45	3.15	3.45	3.00	2.75	3.35	0.17	0.67	0.11	0.01
Marbling score[4]	511	443	474	438	398	470	13.4	0.28	0.002	0.004
REA, cm^2	80.6	79.9	81.8	81.8	82.1	78.9	1.07	0.16	0.99	0.04
Fat, cm	1.48	1.28	1.28	1.24	1.00	1.24	0.06	0.98	0.001	0.01

[1]Treatments: Low (-): low S; Low (+): low S + 10 g·steer^{-1}·d^{-1} vitamin C; Medium (-): medium S; Medium (+): medium S + 10 g·steer^{-1}·d^{-1} vitamin C; High (-): high S; High (+): high S + 10 g·steer^{-1}·d^{-1} vitamin C

[2]Contrast statements: A= vitamin C versus no vitamin C; B = linear effect of sulfur; C = vitamin C within high sulfur

[3]Quality grade: 2: Select$^+$; 3: Choice$^-$; 4: Choice

[4]Marbling scores: slight: 300, small: 400, modest: 500

Table 8. Effect of supplemental vitamin C (10 g·steer^{-1}·d^{-1} vitamin C; VC) on distribution of quality and yield grades of steers fed a low S (0.22%), medium S (0.34%), or high S (0.55%) diet for 149 days

Diet sulfur	Low[1]		Medium[1]		High[1]		Contrast Statement[2]		
Diet vitamin C	-	+	-	+	-	+	A	B	C
Pen/treatment (n)	5	5	5	5	5	5			
USDA QG[3], %									
Prime	10	5	5	0	0	0	1.0	--	1.0
Choice	80	75	95	85	52.6	90	0.72	0.62	0.01
Select	10	20	0	15	42.1	10	0.31	0.15	0.02
Standard	0	0	0	0	5.3	0	--	1.0	0.23
USDA YG[3], %									
1	0	10	0	5	15.8	0	1.0	1.0	0.03
2	45	35	45	35	68.4	50	0.15	0.06	0.24
3	40	50	45	40	15.8	35	0.30	0.05	0.16
4 and 5	15	5	10	20	0	15	0.18	0.24	0.04

[1]Treatments: Low (-): low S; Low (+): low S + 10 g·steer^{-1}·d^{-1} vitamin C; Medium (-): medium S; Medium (+): medium S + 10 g·steer^{-1}·d^{-1} vitamin C; High (-): high S; High (+): high S + 10 g·steer^{-1}·d^{-1} vitamin C

[2]Contrast statements: A= vitamin C versus no vitamin C; B = linear effect of sulfur; C = vitamin C within high sulfur

[3]Quality and yield grades are based on percentages within treatments

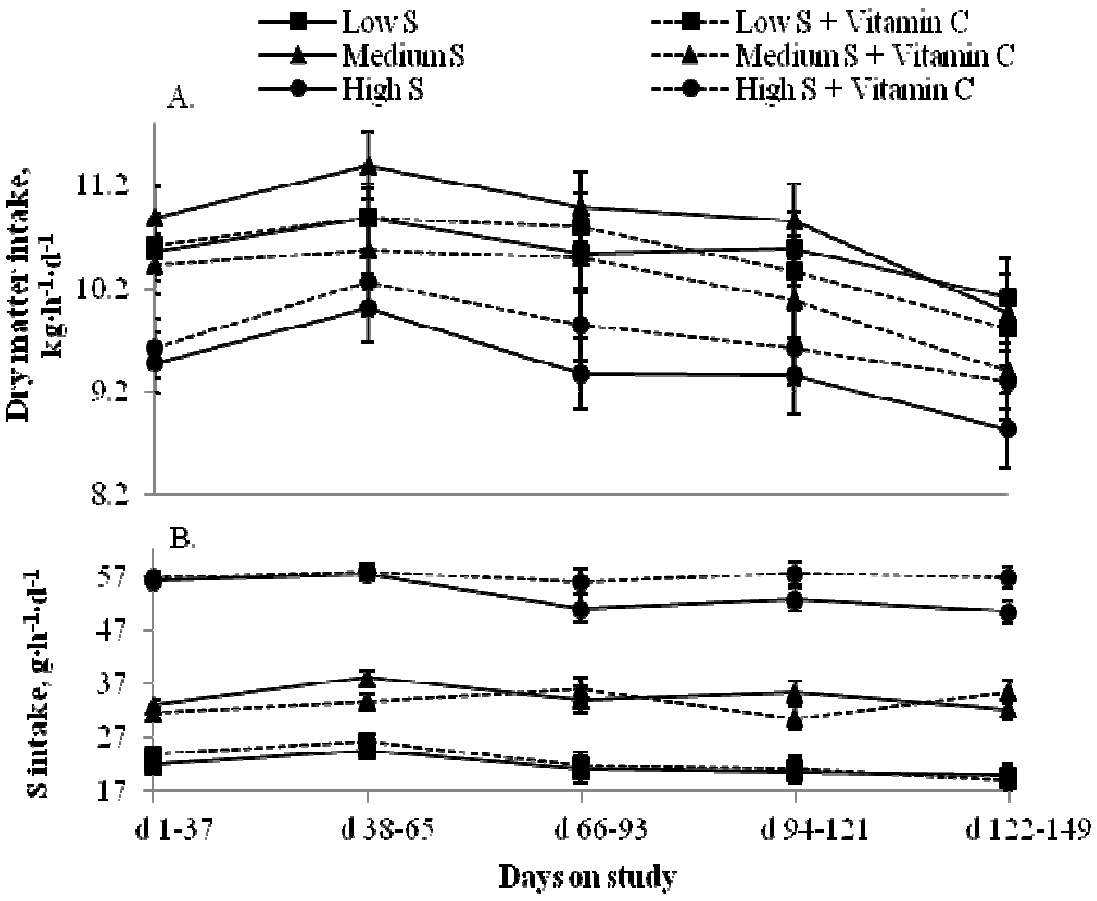

Figure 1. Effect of supplemental vitamin C on dry matter intake (A) and sulfur intake (B) of steers (n = 5 pens per treatment, 1 steer per pen) fed a low (0.22% S,■), medium (0.34% S,▲), or high (0.55% S,●) S diets for 149 days; dry matter intake: month and treatment × month ($P \leq 0.04$); sulfur intake: month and treatment × month ($P < 0.001$). Error bars indicate standard deviations of the mean.

Figure 2.

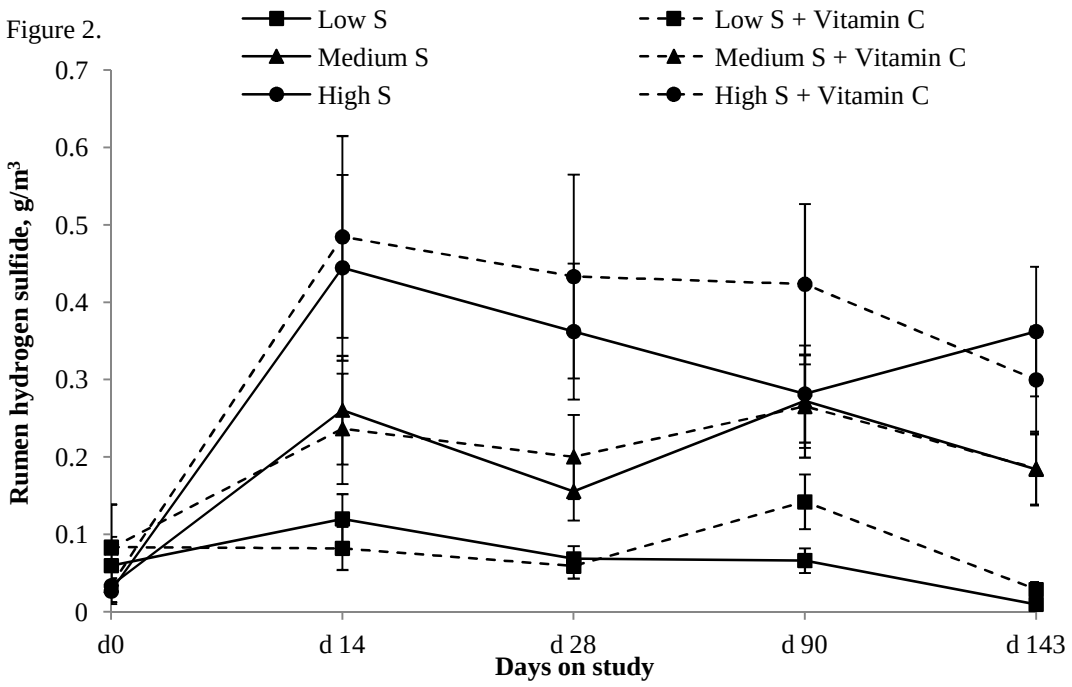

Figure 2. Effect of supplemental vitamin C on rumen hydrogen sulfide concentration of steers (n = 5 per treatment, 1 steer per pen) fed low (0.22% S,■), medium (0.34% S,▲), or high (0.55% S,●) sulfur diets for 143 days; treatment × day interaction (P = 0.001); linear effect of S (P = 0.001). Error bars indicate standard deviations of the mean.

Figure 3.

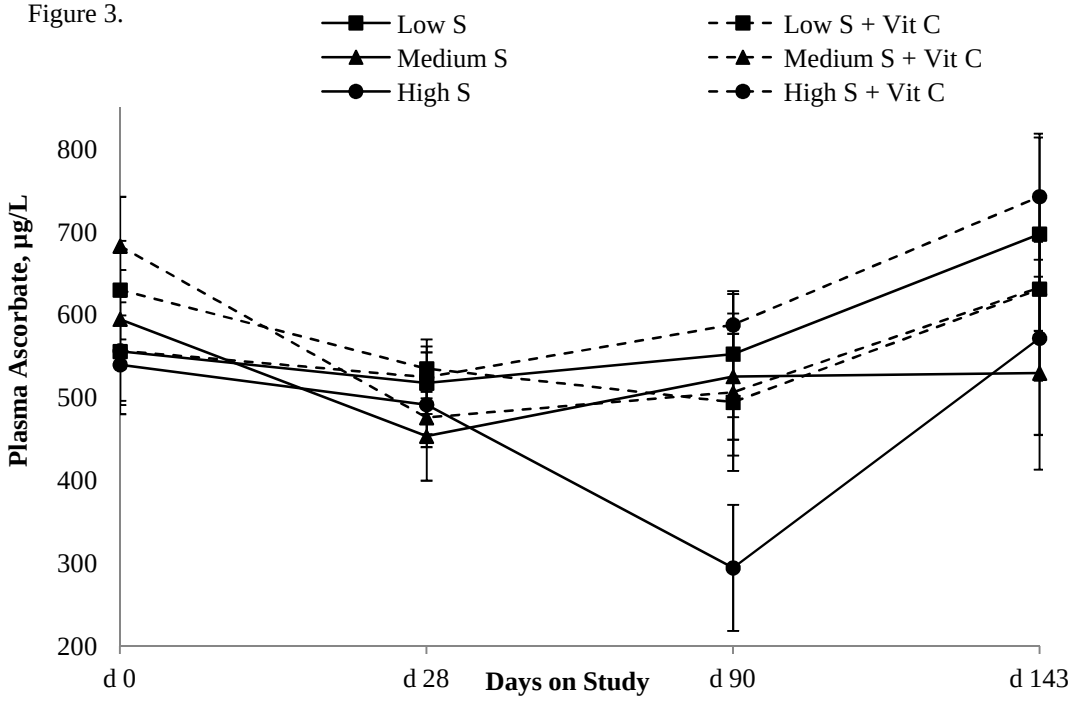

Figure 3. Effect of supplemental vitamin C on plasma ascorbate concentrations of steers (n = 5 per treatment, 1 steer per pen) fed low (0.22% S,■), medium (0.34% S,▲), or high (0.55% S,●) sulfur diets for 143 days; vitamin C versus no vitamin C (*P* = 0.08); vitamin C within the high S diet (*P* = 0.02). Error bars indicate standard deviations of the mean.

CHAPTER 5.

INFLUENCE OF SUPPLEMENTAL VITAMIN C ON POSTMORTEM PROTEIN DEGRADATION AND FATTY ACID PROFILES OF THE *LONGISSIMUS THORACIS* OF STEERS FED VARYING CONCENTRATIONS OF DIETARY SULFUR

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ABSTRACT: The objective was to examine the effects of supplemental vitamin C (**VC**) on postmortem protein degradation and fatty acid profiles of cattle receiving varying concentrations of dietary sulfur (**S**). A *longissimus* muscle was collected from 120 Angus-cross steers assigned to a 3 × 2 factorial, evaluating three concentrations of dietary S (0.22, 0.34, and 0.55%) and two concentrations of supplemental VC (0 or 10 $g \cdot h^{-1} \cdot d^{-1}$). Increasing dietary S and VC supplementation ($P < 0.001$) increased the percent polyunsaturated fatty acids of steaks. Addition of VC tended to increase ($P = 0.09$) both Fe and 2-thiobarbituric acid content of *longissimus thoracis*. Increasing S increased ($P = 0.03$) the proportion of 80-kDa subunit of μ-calpain. Addition of VC within the high S treatment increased ($P = 0.05$) the abundance of 76-kDa subunit of μ-calpain. Increasing S decreased troponin T degradation ($P = 0.07$) and protein carbonylation ($P < 0.01$). Supplemental VC appears to alleviate negative effects of high S on autolysis of μ-calpain and protein degradation.

INTRODUCTION

Distillers grains plus solubles (**DDGS**) are a nutritional and less costly alternative to corn in feedlot diets; however, the concentration of sulfur (**S**) in DDGS remains a limiting factor to its inclusion in cattle diets (Klopfenstein, Erickson, & Bremer, 2007). Diets that contain a high S content are detrimental to animal performance and carcass quality (Pogge & Hansen, 2013; Richter, Drewnoski, & Hansen, 2012; Zinn et al., 1997), specifically decreasing average daily gain, hot carcass weight, and marbling, while also potentially contributing to the development of oxidative stress in the animal (Pogge & Hansen, 2013).

Depletion of body antioxidants hinders ante- and postmortem performance, increasing lipid oxidation and decreasing protein degradation, and subsequently, meat tenderness (Morrissey, Buckley, Sheehy, & Monahan, 1994; Rowe, Maddock, Lonergan, & Huff-Lonergan, 2004a). Because consumer acceptance of meat products is primarily dependent on tenderness and meat favor (Miller et al., 1995; Smith et al., 2009), antioxidants, such as vitamins C (**VC**) and E, are attractive additives to both live animal diets and during meat processing as a means to combat the development of an oxidative environment in both ante- and postmortem muscle, to extend color and lipid stability, to prolong shelf-life (Hood, 1975; Mitsumoto et al., 1991; Rowe, Maddock, Lonergan, & Huff-Lonergan, 2004b). The influence of supplemental rumen-protected VC on protein oxidation in post mortem muscle is not known, specifically when cattle are consuming a high S (or high DDGS) diet.

It has been well documented that increasing the inclusion of distillers grains (both wet and dry) into cattle diets increases polyunsaturated fatty acid (**PUFA**) deposition in meat (Kroger et al., 2010; Mello et al., 2012; Vander Pol, Luebbe, Crawford, Erickson, & Klopfenstein, 2009) and adipose tissue (Aldai et al. 2010; Dugan et al., 2010). However,

greater deposition of PUFA in muscle can present problems for retailers and consumers, as PUFA have a greater propensity to oxidize (Kanner, 1994; Wood et al., 2004). Oxidation of PUFA can limit shelf-life and consumer acceptance of meat products due to the development of off-flavors and the deterioration of meat color (Renerre, Dumont, & Gatellier, 1996; Wood et al., 2004). It was hypothesized that supplemental VC would support a reducing environment within the postmortem muscle to limit the oxidation of proteins and lipids. The objective of this study was to examine the impact of supplemental VC on meat quality, specifically protein degradation and oxidation, and the fatty acid profiles of steers fed varying concentrations of dietary S, which were achieved through the use of two inclusions of DDGS and the addition of sodium sulfate.

MATERIALS and METHODS

Procedures and protocols for the cattle experiment were approved by the Iowa State University Institutional Animal Care and Use Committee (protocol number 7-10-6986-B).

Animals and Experimental Design

Angus-cross calf-fed steers (n = 120) were blocked by initial BW (341 ± 11 kg) and treatments were randomly assigned to a pen of four steers within a block, resulting in five pens per treatment. Cattle were assigned equally to one of six treatments to determine the influence of three concentrations (low, medium, and high) of dietary S (0.22, 0.34, and 0.55% S, respectively) and two concentrations of supplemental VC (0 or 10 $g \cdot h^{-1} \cdot d^{-1}$; Table 1) on meat composition and quality. The VC product (Vitashure C50) is a rumen protected 50% VC product (Balchem Corp., New Hampton, NY). Cattle receiving supplemental VC

consumed an average of 10.3 g VC·h^{-1}·d^{-1}, and daily g of S consumed per steer across the finishing period averaged 22.2, 34.1, and 55.3 g·h^{-1}·d^{-1}, for low S (**LS**), medium S (**MS**), and high S (**HS**), respectively. In this report, treatments will be referred to as the following: LS, LS + VC, MS, MS + VC, HS, and HS + VC. One steer, receiving the HS diet, was removed from study after developing polioencephalomalacia symptoms on d 69, and data from this steer were removed prior to analysis.

More detailed information regarding the live animal portion of this study has been previously reported (Pogge & Hansen, 2013). Steers were slaughtered on d 149 (averaging of the trial when greater than 60% of steers in a pen were estimated by visual appraisal to have at least 1.27 cm of 12^{th} rib fat. At slaughter steers averaged 565 ± 6.8 kg. Steers were slaughtered at a commercial packing facility in Denison, IA (Tyson Fresh Meats). Individual identification was maintained with each carcass following harvest. Carcasses were chilled for 24 h, after which carcasses were ribbed between the 12^{th} and 13^{th} rib and graded, masked to treatment, by representatives of the Tri-County Carcass Futurity (Iowa State University Beef Extension, Lewis, IA) according to USDA standards; however, data was not collected from three carcasses. Following the 24 h chill, a rib steak, approximately a 1.27 cm thick, (across the length and width of the *longissimus thoracis*) was collected from the right side of each carcass (n = 116). Collected *longissimus thoracis* samples were placed into plastic bags with an identification card and transported on ice back to the laboratory at Iowa State University where samples were immediately stored at -20°C until further analysis. Prior to analysis each sample was powdered in a Waring blender (Torrington, CT) using liquid nitrogen to ensure a homogenized sample. A subsample of the powdered muscle was used for myofibrillar protein extraction and mineral analysis, while a separate sample was used for proximate and TBARS

analysis. A sample was taken from the 30 steaks (n = 1 steak per pen, 5 steaks per treatment) designated for fatty acid analysis prior to powdering for myofibrillar protein extraction and mineral analysis, and these 30 steaks were not included in the proximate and TBARS analysis.

Troponin T, µ-Calpain, and Protein Carbonylation Western Blot Analysis

Whole muscle protein extraction was conducted according to the method previously described by Lonergan, Huff-Lonergan, Wiegand, & Kriese-Anderson (2000). Protein concentration was determined according to the method outlined by Lowry, Rosebrough, Farr, & Randall (1951). The gel composition and western blotting for troponin T (n = 114) and µ-calpain (n = 84) were conducted according to a modified method previously reported by Rowe et al. (2004b). Modifications to the procedure by Rowe et al. (2004b) are related to differences in antibody dilutions. The primary antibody for troponin T measurements, monocolonal anti-troponin-T (T6277; clone JLT-12; mouse IgG1 isotype; Sigma-Aldrich, St. Louis, MO) was diluted at 1:10,000, and the primary antibody for µ-calpain analysis was monoclonal anti-µ-calpain (MA3-940; Affinity Bioreagents, Inc., Golden, CO) diluted at1:10,000. A common secondary antibody, anti-mouse IgG peroxidase conjugate (A-2554; Sigma-Aldrich), was diluted at 1:10,000 and 1:20,000 for troponin-T and µ-calpain analysis, respectively. Immunodetection of protein carbonyl group formation was determined (n = 88) according to the manufacturer's instructions using the OxyBlot Protein Detection Kit (catalog # S7150; EMD Millipore, Billerica, MA).

Iron Concentration, Lipid Oxidation, and Proximate Analysis

Approximately 1 g of homogenized *longissimus thoracis* sample (n = 110) was digested in 10 mL of trace metal grade nitric acid through closed vessel digestion in a Mars Xpress microwave (CEM Corporation, Matthew, NC), and brought to a final volume of 100 mL with deionized water. Iron content was determined using inductively coupled plasma optical emission spectrometry (Optima 7000 DV, Perkin Elmer, Waltham, MA), and the iron (**Fe**) concentration of each sample was adjusted to a per gram of wet tissue basis. A bovine liver standard (National Institute of Standards and Technology, Gaithersburg, MA) was included on each session to verify instrument accuracy. A measure of lipid peroxidation of the *longissimus thoracis* sample (n = 83) was determined using the 2-thiobarbituric acid distillation (**TBARS**) method previously described by Tarladgis, Watts, & Younathan (1960). Percent moisture, protein, and fat (n = 85) were determined according to Association of Official Analytical Chemists (2006*a*, chapter 39), Association of Official Analytical Chemists (2006*b*, chapter 39), and Association of Official Analytical Chemists (2006*c*, chapter 39).

Fatty Acid Analysis

Day 2 steaks were prepared for fatty acid analysis by trimming external fat from the *longissimus thoracis* sample (n = 30; 1 steak per pen, 5 steaks per treatment), and lipid was extracted from 2 g of ground muscle tissue (wet) according to the method previously described by Folch, Lees, & Stanley (1957). The total lipid extracted from each steak was calculated as a percentage of the original 2 g of wet tissue. The extracted lipid was then esterified using the acetyl chloride/methanol method of Christie (1972). The extracted lipid

was standardized to achieve 40 mg esterified lipid, which was accurately measured into an airtight Teflon capped tube. Each sample was evaporated under nitrogen gas, after which 1 mL of methanol and 100 µL of acetyl chloride were added to each tube, and samples were purged with nitrogen gas before capping. Samples were vortexed and heated at 80°C for 1 h (heating block), and additionally vortexed every 20 min. After heating for 1 h, samples were cooled to room temperature. To each tube 5 mL of 4% potassium carbonate and 2 mL of hexane were added, samples were purged with nitrogen gas, and vortexed. Samples were centrifuged at 1700 rpm for 10 min. The esterified lipid layer (top layer) was removed and transferred to a gas chromatography vile, purged with nitrogen gas to limit fatty acid oxidation, and stored at -20°C until analysis. Gas chromatography (**GC;** model 3900; Varian Analytical Instruments, Walnut Creek, CA) was utilized to analyze samples for fatty acid profiles using the method previously described by Richter et al. (2012), and 1 µL of fatty acid methyl esters (**FAME**) sample was injected into the GC with split ratio 99. Fatty acid methyl esters standards gas-liquid chromatography (**GLC**) 68D, GLC 79, GLC 81, and GLC461 (Nu-Chek Prep, Inc., Elysian, MN) were used to determine peak identification and quantification (Kramer et al., 2008). The percent saturated fatty acids (**SFA**), monounsaturated fatty acids (**MUFA**), PUFA, omega 3 fatty acids, and omega 6 fatty acids, and the ratio of PUFA-to-SFA and omega 3-to-6 fatty acids were calculated. Atherogenic index (**AI**) was calculated according to the equation described by Ulbricht & Southgate (1991):

$$\frac{((C12:0) + (4 \times C14:0) + (C16:0))}{\%\ MUFA + \%\ PUFA}$$

An index of the enzyme responsible for the desaturation of two common SFA C16:0 and C18:0, Δ-9-desaturase, was calculated according to the following equations, previously reported by Malau-Aduli et al. (1998):

Δ-9-desaturase (16) index: C16:1n7 / (C16:0 + C16:1n7) *100

Δ-9-desaturase (18) index: C18:1c9 / (C18:0 + C18:1c9) *100

Statistical analysis

Data were analyzed by ANOVA as a complete randomized block design using the MIXED procedure of SAS version 9.2 (SAS Institute Inc., Cary, NC). The model for the analysis of all data included the fixed effect of treatment, while block was the random effect. Pen was the experimental unit for all analysis (n = 5 per treatment). Cook's D outlier test was used to determine outliers, which were subsequently removed from data analysis. Five single degree of freedom contrast statements were used to compare: A) VC versus no VC, B) linear effect of S, C) VC within LS corn diet (0.22% S), D) VC within 40% DDGS (MS and HS) diets, and E) VC within HS (0.56% S). Probability values less or equal to 0.05 were considered significant, while *P* values >0.05 and ≤ 0.10 were considered tendencies.

RESULTS

μ-Calpain Autolysis

Data concerning the autolysis of the protease μ-calpain, measured 2-d postmortem, are presented in Figure 1. As the concentration of S in the diet increased, a linear increase (*P* = 0.03) in the quantity of the 80 kDa catalytic subunit of μ-calpain was observed. However, the addition of VC to the high S diet tended (*P* = 0.09) to decrease the presence of the 80 kDa

subunit. This indicates that the VC created conditions more favorable for autolysis. The inclusion of VC to any level of dietary S tended to increase (P = 0.09) the portion of the 76 kDa catalytic subunit of μ-calpain. However, this effect is largely driven by the medium and high S treatments, where VC supplementation increased (P = 0.03) the presence of the 76 kDa subunit from 41.7% to 52.7% and 38.1% to 55.6% (± 6.3), for the MS, MS+VC, HS, and HS+VC, respectively.

Troponin T Degradation and Protein Carbonylation

Troponin T degradation and protein carbonylation data, measured 2-d postmortem, are presented in Figures 2 and 3, respectively. Troponin T degradation tended (P = 0.07) to decrease with increasing dietary S. This tendency is primarily driven by the high S treatments, which demonstrated approximately 7 to 12% less troponin T degradation than the low S and medium S treatments. Vitamin C supplementation did not affect (P = 0.13) troponin T degradation or protein carbonylation (P = 0.13). A linear decrease (P = 0.007) in protein carbonylation was observed with increasing concentrations of dietary S in cattle diets.

Proximate Analysis

Results of the proximate analysis of *longissimus thoracis* samples are presented in Table 2. *Longissimus thoracis* from cattle consuming greater concentrations of dietary S had a greater (P = 0.001) percentage of moisture. Increasing dietary S resulted in a lesser (P = 0.001) percentage of intramuscular fat, primarily being driven by the depression in lipid content in *longissimus thoracis* from cattle receiving high S diets. The addition of VC to the

HS treatment tended ($P = 0.07$) to decrease the percentage of protein in the *longissimus thoracis.*

Iron Concentration and Lipid Oxidation

Iron and TBARS data are presented in Table 3. Cattle consuming supplemental VC, regardless of dietary S, tended ($P = 0.09$) to have greater Fe content in the *longissimus thoracis*. This increase was most notable in the low S treatments, where VC supplementation increased ($P = 0.008$) Fe deposition from 12.8 to 14.7 (± 0.46) mg/kg. No effect ($P = 0.86$) of dietary S on Fe concentration in the *longissimus thoracis* was observed. The inclusion of VC, regardless of dietary S, tended to increase ($P = 0.09$) TBARS values, most notably within the low S diets ($P = 0.04$; 0.29 and 0.33 µM MDA ± 0.01, for LS and LS+VC, respectively) and high S diets ($P = 0.01$; 0.27 and 0.33 µM MDA ± 0.01, for HS and HS+VC, respectively).

Fatty acid percentages and ratios

The *longissimus thoracis* fatty acid percentages and calculated ratios and individual fatty acids (mg fatty acids/100 g fresh meat) are presented in Tables 4 and 5, respectively. The concentration of SFA was not different due to treatment ($P \geq 0.66$). However, the concentration of PUFA in the steaks increased with VC supplementation ($P = 0.01$) and as dietary S increased ($P < 0.001$), thus increasing ($P < 0.001$) the ratio of PUFA-to-SFA. The increase in PUFA-to-SFA due to dietary S and VC supplementation may be related to a general decrease ($P \leq 0.01$) in several saturated fatty acids, specifically C12:0, C14:0, C16:0, and C17:0. The addition of VC, regardless of dietary S concentration, decreased ($P \leq 0.02$)

C16:0 and C18:0, with the differences primarily being driven by the effect of VC supplementation within the low S and high S treatments.

Supplementation of VC and increasing the concentration of S in the diet favorably decreased ($P \leq 0.02$) the AI. This decrease is likely related to the decreased ($P \leq 0.01$) concentrations of C12:0, C14:0, C16:0, and C17:0 as dietary S concentration increased, and the increase in ($P < 0.001$) PUFA concentration with VC supplementation and increasing dietary S. Increasing concentrations of S in the diet increased ($P < 0.001$) the ratio of omega 6-to-omega 3 fatty acids. The addition of VC to the low S treatment increased ($P = 0.004$) the omega 3 fatty acid concentration, primarily due to an increase ($P = 0.002$) in C20:5n3 in the LS+VC compared to LS.

Increasing dietary S tended ($P = 0.10$) to decrease MUFA content of steaks. Vitamin C supplementation, regardless of dietary S concentration, did not alter the Δ-9-desaturase (16) index ($P = 0.15$), however, VC supplementation within the medium and high S treatments increased ($P = 0.02$) the Δ-9-desaturase (16) index. Increasing dietary S decreased ($P = 0.04$) the Δ-9-desaturase (16) index, which is primarily being driven by the medium S treatments. As dietary S increased and VC was supplemented the Δ-9-desaturase (18) index tended to increase ($P \leq 0.09$), primarily driven by the high S treatment. Within the high S treatment, the addition of VC increased ($P < 0.01$) the Δ-9-desaturase (18) index.

DISCUSSION

Oxidative conditions, developed ante-mortem, can have negative impacts on the development of meat quality, specifically impacting tenderness and shelf-life (Decker, Xiong, Calvert, Crum, & Blanchard, 1993; Morrissey et al., 1994; Miller et al., 1995; Huff-

Lonergan, Zhang & Lonergan, 2010). The current study was designed to determine the influence of S-induced oxidative stress on postmortem oxidative conditions within the muscle and subsequent impact on protein degradation and fatty acid profiles. Steers consuming a high S (0.55% S) diet experienced some oxidative stress, indicated by an increase in oxidized liver glutathione (28%) compared to 7% oxidized glutathione in the high S steers supplemented with VC (Pogge & Hansen, 2013). Additionally, a decrease in plasma total antioxidant capacity was observed throughout the finishing period, especially noted in the high S treatment (Pogge & Hansen, 2013).

Previous research indicates the exposure of µ-calpain to an oxidative environment may interfere with its ability to complete autolysis and exert proteolytic activity (Guttmann & Johnson, 1998; Lametsch, Lonergan, & Huff-Lonergan, 2008; Rowe et al., 2004b). Because VC is a potent antioxidant, it was hypothesized that the reducing power contributed by the vitamin would provide protection for the active site of µ-calpain to maintain the reduced form for a longer period of time, thus allowing for proteolysis. In the present study, VC supplementation to cattle consuming the high S diet increased the percentage of the 76 kDa subunit of µ-calpain by approximately 18% compared to the HS treatment. Within the high S treatment, the addition of VC recovered the percentage of the 76 kDa subunit (55%) to values comparable with those of the low S treatments (54% and 52%, LS and LS+VC, respectively). While µ-calpain enzyme activity was not evaluated in the present study, the increase in µ-calpain autolysis of the HS+VC treatment may indicate the importance of VC to the maintenance of a reducing environment in postmortem muscle.

Troponin T is especially susceptible to degradation by µ-calpain making it an excellent marker for protein degradation within the muscle (Ho, Stromer, & Robson, 1994;

Huff-Lonergan et al., 1996). In the present study, increasing dietary S tended to decrease troponin T degradation, specifically in the high S treatments. This may indicate that less autolysis occurred in the first 2 d postmortem. These results suggest diets exceeding 0.34% dietary S may result in negative impacts on the tenderness and eating quality of the final beef products.

Similarly, Rowe et al. (2004a) used irradiation to induce oxidation and observed less extensive calpain autolysis and limited degradation of troponin T at d 3, 7, and 14 post irradiation of beef steaks, which translated to an increase in shear force values in the irradiated steaks. In the present study, differences due to dietary S and VC supplementation may have been more evident had samples been aged beyond 2-d postmortem. Previous research indicates a positive relationship between the extent of troponin T degradation and tenderness scores (Huff-Lonergan et al., 1996; Lonergan et al., 2001). In the present study, degradation of troponin T was negatively associated (-0.53; $P < 0.001$) with the percentage of the 80 kDa subunit of µ-calpain and positively associated (0.63; $P < 0.001$) with the 76 kDa subunit of µ-calpain. These results tie activation of calpain together with postmortem proteolysis in muscle.

The addition of antioxidants to combat the development of oxidative conditions in the postmortem muscle has been thoroughly investigated. However, researchers have primarily been concerned with vitamin E, as this vitamin has a greater storage capacity and half-life within the body when compared to VC. Rowe et al. (2004a) were interested in the ability of dietary vitamin E to modulate the development of an oxidative environment, induced by exposing beef steaks to irradiation. At 2-d post-irradiation, increases in troponin T degradation and decreases in protein oxidation were observed in the irradiated steaks from

cattle supplemented with vitamin E. These results suggest the supplementation of a lipid-soluble antioxidant may be beneficial to inhibit protein oxidation in postmortem muscle, and thereby create conditions favorable for proteolysis and tenderization. While vitamin E was not evaluated in the present study, it is well established that VC contributes electrons to aid in the regeneration of the reduced form of vitamin E (Tanaka, Hasimoto, Tokumaru, Iguchi, & Kojo, 1997). However, in the present study, VC supplementation did not have an effect on troponin T degradation. The differences in results of the studies may be related to the greater storage capacity of muscle for vitamin E in membranes and lipid depots compared to the limited storage VC in the extracellular matrix.

The interaction of muscle proteins with reactive oxygen species can result in posttranslational modifications, such as the irreversible formation of carbonyl groups on protein side-chains (Stadman and Levine, 2000). Due to the increased ratio of oxidized-to-reduced glutathione of the HS (28%) cattle compared to the VC supplemented HS cattle previously observed (7%; Pogge and Hansen, 2013), it was hypothesized that a greater inclusion of dietary S would increase the protein carbonylation in the meat; however, our hypothesis was disproved as protein carbonylation decreased as dietary S increased. Dissimilarly, Rowe et al. (2004a) reported total protein carbonyls increased in steaks exposed to an oxidative environment (irradiation) compared to the non-irradiated steaks.

Distillers grain products are often variable in fat content, ranging from 4 to 12% fat, and Vander Pol et al. (2009) suggested the lipid associated with DDGS diets may be more protected against hydrogenation in the rumen compared to diets supplemented with corn oil, resulting in a greater percentage of unsaturated fatty acids absorbed from the small intestine and incorporated into body lipid depots. Kroger et al. (2010) reported a greater PUFA

deposition in the meat of cattle consuming 40% DDGS compared to 20% DDGS, while no differences in SFA content or marbling scores were observed. Similarly, in the present study PUFA deposition increased by approximately 1.5 to 2.25% in the medium and high S treatments (40% DDGS inclusion) compared to the low S treatment (18% DDGS). While total fatty acid profiles of the diets were not analyzed, the increase in PUFA concentration of the steaks may be attributed to the traditionally greater percentage of PUFA present in DDGS products. Regardless of dietary S inclusion, the addition of VC to the diet magnified the increase in the percent PUFA in the muscle; however, the reason for this increase is unknown. One possible explanation for the increase in PUFA concentration may be related to less intramuscular fat content as a result of S inclusion (Pogge & Hansen, 2013), specifically within the high S treatment, which may have increased the relative proportion of cell membrane lipid in the samples.

In recent decades, research data indicate that the consumption of more omega 3 fatty acids and fewer SFA can reduce the risk of developing cardiovascular diseases (Hu, Manson, & Willett, 2001). In the present study, the supplementation of VC in the low S diet decreased the ratio of omega 6-to-omega 3 fatty acids (14.56% and 12.13%, LS and LS+VC, respectively). This decrease is likely attributed to an increase in C20:5n3, potentially indicating the supplementation of VC may provide a way for producers to improve the lipid profile of beef to provide health benefits for consumers. The addition of VC to the diets contributed to a decrease in C16:0 and C18:0 content in intramuscular adipose tissue. Kroger et al. (2010) reported similar fatty acid profiles (decreased C16:0 and C18:0) when cattle were consuming a 20% or 40% DDGS diet. Additionally, Oohashi, Takizawa, & Morita (1999) reported a decrease in intramuscular SFA (C16:0 and C18:0) when Japanese Black

cattle were supplemented with VC throughout the finishing period. It is important to note, as far as the authors are aware, there has been no previous research examining the influence of VC supplementation to cattle finished in the United States.

Because the desaturation of fatty acids requires reducing equivalents (Oshino, Imai, & Sato, 1971), cattle experiencing oxidative stress may be depleting the availability of reducing equivalents required for the desaturation process. The antioxidant capacity of VC may provide a sparing mechanism for reducing equivalents, specifically within the high S treatments, to increase the availability for desaturase activity. While desaturase activity was not evaluated in the present study, the indices of Δ-9-desaturase (C16 and C18) were calculated. The Δ-9-desaturase (C16 and C18) indices have been used as an index of Δ-9-desaturase activity (Smith et al., 2002). Interestingly, the supplementation of VC within the 40% DDGS treatments (medium S and high S) increased the calculated Δ-9-desaturase (16) index, and VC inclusion within the high S treatment increased the Δ-9-desaturase (18) index. The differences in fatty acid profiles and calculated ratios and indices suggest that VC is having an impact on cellular metabolism; however, the differences are inconsistent between the low S (corn-based) and high S (DDGS-based) diets.

There are several factors within the present study that may be contributing to the oxidation of lipids, specifically the greater percentage fat from the DDGS and muscle Fe content, which can act as a pro-oxidant in vivo. Several authors have reported a greater incidence of lipid oxidation, measured via TBARS, in both ground beef and steaks from cattle consuming increasing concentrations of WDGS and DDGS (de Mello et al., 2012; Gill, VanOverbeke, Depenbusch, Drouillard, & DiCostanzo, 2008; Kroger et al., 2010). In the present study, increasing dietary S did not impact lipid oxidation in early postmortem meat (2

d); however, VC supplementation increased TBARS values within the low and high S treatments. It was hypothesized that the antioxidant capabilities of VC would decrease lipid oxidation in treatments supplemented with the vitamin; however, the results contradict this prediction, and given the very small differences among treatments, the differences seem unlikely to be biologically relevant.

In conclusion, feeding high S diets (greater than 0.34% S) to feedlot steers decreased the percent of the 76 kDa subunit of μ-calpain and decreased troponin T degradation. Together these factors may decrease the overall tenderness and eating quality of the final meat product. The benefits observed within the high S treatment may have been at least partially in response to the oxidative stress induced by the high S diet. Within the high S treatment, VC may have potential to increase both the quality and tenderness of the final meat products, possibly by contributing to the reducing environment protecting calpain from oxidation. Within the low and medium S treatments, VC supplementation displayed differing effects. The low S treatment experienced little to no effect of VC on protein degradation or carbonylation; however, VC decreased the ratio of omega 6-to-omega 3 fatty acids and Fe deposition in steaks. Within the medium S treatment, VC supplementation recovered the 76 kDa subunit of μ-calpain, to values comparable with low S. Because distillers grains are often high in dietary S, feeding VC to cattle may allow producers to enjoy the economic benefits of a distillers grains-based diet while still maintaining the quality and tenderness expected by consumers.

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Table 1. Ingredient composition and chemical analysis of finishing diets (% DM basis)

Item	Low sulfur[1,2]	Medium sulfur[1,2]	High sulfur[1,2,6]
Corn	69.7	48.0	48.0
Corn dried distiller's grains[3]	18.0	40.0	38.9
Chopped hay	9.0	9.0	9.0
Limestone	2.0	2.0	2.0
Salt	0.31	0.31	0.31
Vitamin A premix[4]	0.10	0.10	0.10
Trace mineral premix[5]	0.035	0.035	0.035
Rumensin90[6]	0.016	0.016	0.016
Sodium sulfate[7]	--	--	1.11
Urea	0.80	--	--
Analyzed composition			
CP, %	14.3	16.6	16.6
NEg, Mcal/kg DM	1.3	1.3	1.3
Sulfur[8], %	0.22	0.34	0.55
Calculated composition			
% Lipid	4.70	6.06	5.95

[1]Treatments: low sulfur (0.22% S), medium sulfur (0.34% S), high sulfur (0.55% S)

[2]Vitashure C, provided by Balchem Corp., replaced dry distillers grains plus solubles at 0.215% DM to achieve 10 g of vitamin C per steer per day; vitamin C intake averaged 10.3 g per steer per day

[3]Five loads of dry distillers grains plus solubles from Lincoln Way Energy (Nevada, IA) were used during the trial with sulfur concentrations of 0.72%, 0.67%, 0.70%, 0.79%, and 0.55%

[4]Vitamin A premix contained 4,400,000 IU/kg

[5]Provided per kg of diet: 30 mg Zn as $ZnSO_4$; 20 mg Mn as $MnSO_4$; 0.5 mg I as $Ca(IO_3)_2(H_2O)$; 0.1 mg Se as Na_2SeO_3; 10 mg Cu as $CuSO_4$; and 0.1 mg Co as $CoCO_3$

[6]Provided Rumensin90 at 27 g/ton diet (donated by Elanco Animal Health)

[7]Sodium sulfate added to the diet, at the expense of dry distiller grains plus solubles, to increase the percent sulfur of the medium sulfur diet by 0.36% sulfur

[8]Percent sulfur for low, medium, and high diets are based on repeated measures analysis of samples collected over the 149 d study

Table 2. Proximate analysis of the *longissimus thoracis* collected from steers supplemented with vitamin C (10 g·steer^{-1}·d^{-1} vitamin C) on a low sulfur (0.22%), medium sulfur (0.34%), or high sulfur (0.55%) diet

Diet sulfur	Low[1]		Medium[1]		High[1]			Contrast Statement[2]				
Diet vitamin C	-	+	-	+	-	+	SEM	A	B	C	D	E
Pen (steers)	5(13)	5(13)	5(15)	5(15)	5(14)	5(15)						
Moisture, %	71.9	72.2	72.1	72.4	73.2	73.0	0.29	0.55	<0.01	0.40	0.87	0.62
Fat, %	5.40	5.03	5.48	5.05	3.56	4.29	0.38	0.93	<0.01	0.47	0.69	0.19
Protein, %	22.3	22.1	21.5	22.0	22.3	21.9	0.10	0.99	0.78	0.21	0.38	0.07

[1]Treatments: Low (-): low sulfur; Low (+): low sulfur + 10 g·steer^{-1}·d^{-1} vitamin C; Medium (-): medium sulfur; Medium (+): medium sulfur + 10 g·steer^{-1}·d^{-1} vitamin C; High (-): high sulfur; High (+): high sulfur + 10 g·steer^{-1}·d^{-1} vitamin C

[2]Contrast statements: A = vitamin C versus no vitamin C; B = linear effect of sulfur; C = vitamin C within the low sulfur corn diet; D = vitamin C within 40% dry distiller grains plus solubles (medium and high sulfur) diets; E = vitamin C within high sulfur diet

Table 3. Effect of vitamin C supplementation on iron (Fe) content and thiobarbituric acid-reactive substances (TBARS) in the *longissimus thoracis* collected from steers fed a low sulfur (0.22%), medium sulfur (0.34%), or high sulfur (0.55%) diet

Diet sulfur	Low[1]		Medium[1]		High[1]			Contrast Statement[2]				
Diet vitamin C	-	+	-	+	-	+	SEM	A	B	C	D	E
Pen(steers)	5(18)	5(17)	5(19)	5(20)	5(18)	5(18)						
Fe, mg/kg	12.8	14.7	12.7	13.8	14.0	13.1	0.46	0.09	0.85	<0.01	0.88	0.24
Pen(steers)	5(13)	5(13)	5(12)	5(17)	5(12)	5(16)						
MDA[3], mg/kg	0.29	0.33	0.29	0.24	0.27	0.33	0.01	0.09	0.79	0.04	0.55	<0.01

[1]Treatments: Low (-): low sulfur; Low (+): low sulfur + 10 g·steer^{-1}·d^{-1} vitamin C; Medium (-): medium sulfur; Medium (+): medium sulfur + 10 g·steer^{-1}·d^{-1} vitamin C; High (-): high sulfur; High (+): high sulfur + 10 g·steer^{-1}·d^{-1} vitamin C

[2]Contrast statements: A = vitamin C versus no vitamin C; B = linear effect of sulfur; C = vitamin C within the low sulfur corn diet; D = vitamin C within 40% dry distillers grains plus solubes (medium and high sulfur) diets; E = vitamin C within high sulfur diet

Table 4. Effect of vitamin C on fatty acid percentages and ratios of the *longissimus thoracis* collected from steers consuming a low sulfur (0.22%), medium sulfur (0.34%), or high sulfur (0.55%) diet

Diet sulfur	Low[1]		Medium[1]		High[1]			Contrast Statement[2]				
Diet vitamin C	-	+	-	+	-	+	SEM	A	B	C	D	E
Pen(steers)	5(5)	5(5)	5(5)	5(5)	5(5)	5(5)						
SFA, %[3]	48.29	48.60	47.58	47.43	48.43	47.61	1.191	0.83	0.82	0.84	0.70	0.66
MUFA, %[4]	42.35	43.28	42.25	42.35	41.39	40.86	1.152	0.83	0.10	0.48	0.82	0.72
PUFA, %[5]	5.92	5.93	7.44	7.58	7.50	8.87	0.230	0.01	<0.01	0.97	<0.01	<0.01
PUFA:SFA	0.115	0.123	0.165	0.160	0.153	0.169	0.0084	0.27	<0.01	0.40	0.44	0.16
n3, %[6]	0.327	0.442	0.479	0.466	0.438	0.441	0.0283	0.11	0.11	<0.01	0.85	0.94
n6, %[7]	5.43	5.32	6.82	6.96	6.88	8.30	0.226	0.01	<0.01	0.73	<0.01	<0.01
n6:n3	14.56	12.13	15.15	15.11	15.86	18.87	0.877	0.78	0.001	0.05	0.08	0.02
Δ-9 desaturase (16) index[8]	11.58	11.06	10.69	11.71	10.10	10.95	0.445	0.15	0.04	0.36	0.02	0.11
Δ-9 desaturase (18) index[9]	72.01	72.23	72.34	72.27	68.94	72.46	0.882	0.09	0.08	0.85	0.05	<0.01
AI[10]	0.756	0.640	0.646	0.629	0.652	0.607	0.0277	<0.01	0.02	<0.01	0.16	0.17
Other, %[11]	2.01	2.19	2.32	2.63	2.28	1.85	0.311	0.93	0.74	0.68	0.86	0.35
Lipid, %[12]	8.43	6.14	6.78	6.73	6.21	5.51	0.65	0.05	0.03	0.02	0.53	0.42

[1]Treatments: Low (-): low sulfur; Low (+): low sulfur + 10 g·steer^{-1}·d^{-1} vitamin C; Medium (-): medium sulfur; Medium (+): medium sulfur + g·steer^{-1}·d^{-1} vitamin C; High (-): high sulfur; High (+): high sulfur + 10 g·steer^{-1}·d^{-1} vitamin C

[2]Contrast statements: A = vitamin C versus no vitamin C; B = linear effect of sulfur; C = vitamin C within the low sulfur corn diet; D = vitamin C within 40% dry distillers grains plus solubles (medium and high sulfur) diets; E = vitamin C within high sulfur diet

[3]Saturated fatty acid calculation, sum of: C10:0, C12:0, C13:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C22:0, C23:0, C24:0

[4]Monounsatruated fatty acid calculation, sum of: C14:1n5, C16:1n7, C17:1n9, C18:1t6 & t9, C18:1t10, C18:1t11, C18:1t12, C18:1t15, C18:1c9, C18:1c11, C18:1c12, C18:1c13, C20:1n11

[5]Polyunsaturated fatty acid calculation, sum of: C18:2n6, C18:3n3, C18:3n6, C20:2n6, C20:3n6, C20:3n3, C20:4n6, C20:5n3, C22:5n3, C22:6n3, c9-t11 CLA

[6]Omega 3 fatty acid calculation, sum of: C18:3n3, C20:3n3, C20:5n3, C22:5n3, and C22n3

[7]Omega 6 fatty acid calculation, sum of: C18:2n6, C18:3n6, C20:2n6, C20:3n6, and C20:4n6

[8]Δ-9-desaturase (16) index is calculated: C16:1n7 / (C16:0 + C16:1n7) *100

[9]Δ-9-desaturase (18) index is calculated: C18:1n9 / (C18:0 + C18:1n9) *100

[10]Atherogenic index is calculated: ((C12:0 + (4 * C14:0) + C16:0) / (% MUFA + % PUFA))

[11]Other indicates the percent of unidentified peaks

[12]Percent lipid of steaks from fatty acid extraction (n = 30; 5/treatment)

Table 5. Impact of vitamin C on individual fatty acids (mg fatty acids/100 g fresh meat) of the *longissimus thoracis* collected from steers consuming a low sulfur (0.22%), medium sulfur (0.34%), or high sulfur (0.55%) diet

Diet sulfur	Low[1]		Medium[1]		High[1]			Contrast Statement[2]				
Diet vitamin C	-	+	-	+	-	+	SEM	A	B	C	D	E
Pen(steers)	5(5)	5(5)	5(5)	5(5)	5(5)	5(5)						
10:0	1.84	2.75	2.13	2.17	1.76	1.96	0.429	0.20	0.25	0.11	0.73	0.67
12:0	3.32	3.34	2.51	2.99	1.77	2.18	0.561	0.43	0.01	0.97	0.34	0.56
14:0	207.8	186.0	150.1	159.7	122.0	109.5	24.47	0.65	<0.01	0.51	0.94	0.71
14:1n5	36.38	31.83	30.94	38.67	29.08	24.59	6.967	0.92	0.18	0.63	0.75	0.54
15:0	33.28	29.57	22.48	21.81	20.06	14.18	4.056	0.26	<0.01	0.50	0.37	0.26
16:0	2150.2	1410.0	1577.6	1488.7	1306.0	1149.6	169.02	0.02	<0.01	0.004	0.04	0.50
16:1n7	290.3	179.4	189.8	192.7	148.7	135.0	29.18	0.06	<0.01	0.01	0.81	0.68
17:0	109.8	94.6	71.6	68.9	62.4	49.8	13.02	0.24	<0.01	0.35	0.43	0.37
17:1n9	71.51	56.73	50.14	29.85	37.86	34.31	8.68	0.04	<0.01	0.21	0.09	0.71
18:0	1038.4	761.5	939.7	888.7	999.5	680.6	97.70	0.004	0.43	0.02	0.03	0.02
18:1c9	2870.8	1972.5	2348.0	2336.6	2278.3	1701.7	281.34	0.03	0.09	0.03	0.24	0.11
18:1c11	8.85	7.37	8.15	8.03	9.86	6.85	1.271	0.13	0.82	0.35	0.21	0.11
18:1c12	24.37	19.07	19.03	21.19	16.28	12.97	3.216	0.39	0.02	0.26	0.84	0.42
18:1c13	3.51	4.42	4.99	6.08	7.34	3.10	0.887	0.27	0.20	0.45	0.07	0.001
18:1t10	17.56	22.73	11.28	12.13	24.72	5.93	4.24	0.20	0.36	0.37	0.03	0.004
18:1t11	282.0	296.6	184.6	183.5	227.1	176.0	37.34	0.66	0.05	0.78	0.45	0.31
18:1t12	2.71	2.45	1.99	3.47	8.81	3.76	1.691	0.26	0.01	0.89	0.21	0.03
18:1t15	61.68	59.81	54.06	53.10	48.20	41.54	5.153	0.43	<0.01	0.80	0.40	0.31
18:2n6	364.5	272.3	419.4	407.7	379.6	379.3	41.81	0.29	0.23	0.11	0.88	1.0
CLA[3]	10.13	9.99	9.99	10.48	11.13	5.22	1.512	0.14	0.19	0.95	0.08	0.01
18:3n3	23.22	18.04	23.18	23.37	20.48	15.79	2.672	0.14	0.22	0.19	0.37	0.20
20:0	0.913	4.860	2.571	3.195	2.705	1.082	1.0959	0.26	0.32	0.02	0.62	0.28
20:1c11	3.79	4.55	3.68	4.73	3.84	2.78	0.930	0.72	0.28	0.54	0.99	0.37
20:3n6	13.49	11.07	13.50	13.65	12.76	11.85	1.345	0.25	0.86	0.13	0.73	0.59

20:4n6	40.93	35.13	42.26	39.66	34.38	41.51	2.443	0.82	0.80	0.10	0.33	0.06
20:5n3	0.738	2.704	2.108	1.230	1.524	1.451	0.405	0.30	0.54	0.002	0.23	0.90
22:5n3	5.92	7.72	6.63	6.79	6.12	6.66	1.18	0.29	0.66	0.17	0.73	0.72
24:0	1.80	2.52	2.19	1.75	1.92	1.38	0.766	0.88	0.47	0.48	0.49	0.60

[1]Treatments: Low (-): low sulfur; Low (+): low sulfur + 10 g·steer^{-1}·d^{-1} vitamin C; Medium (-): medium sulfur; Medium (+): medium sulfur + 10 g·steer^{-1}·d^{-1} vitamin C; High (-): high sulfur; High (+): high sulfur + 10 g·steer^{-1}·d^{-1} vitamin C

[2]Contrast statements: A = vitamin C versus no vitamin C; B = linear effect of sulfur; C = vitamin C within the low sulfur corn diet; D = vitamin C within 40% dry distiller's grains plus solubles (medium and high sulfur) diets; E = vitamin C within high sulfur diet

[3]CLA, Conjugated linoleic acid (mixture of unseparated isomers)

Figure 1.

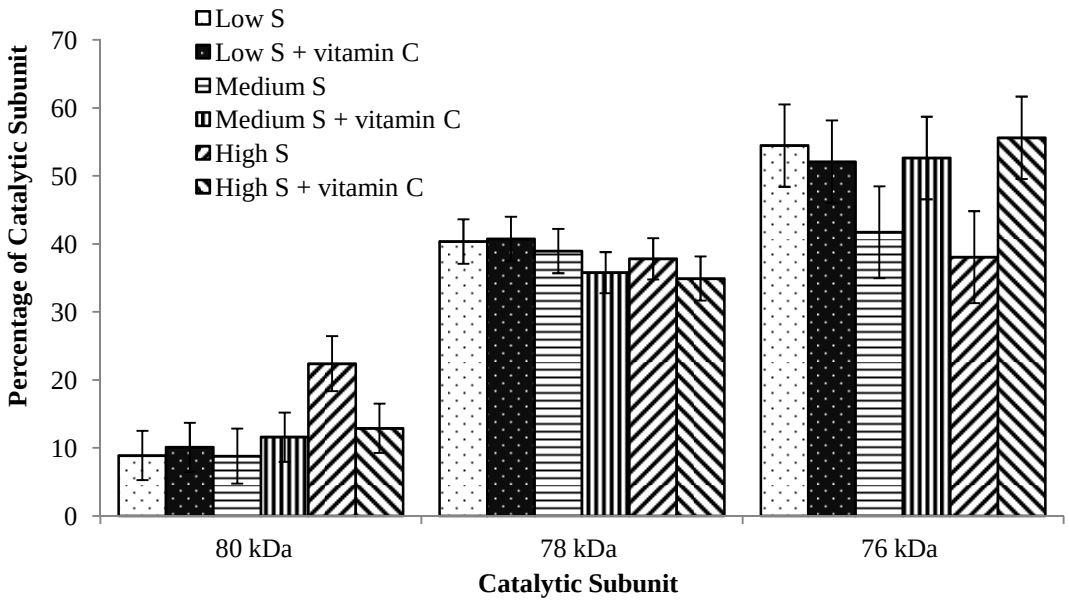

Figure 1. Impact of a rumen-protected supplemental vitamin C source on μ-calpain autolysis at 2-d postmortem in the *longissimus thoracis* collected from steers consuming a low sulfur (S; 0.22%), low S + 10 g·steer^{-1}·d^{-1} vitamin C, medium S (0.34%), medium + 10 g·steer^{-1}·d^{-1} vitamin C, high S (0.55%), high S + 10 g·steer^{-1}·d^{-1} vitamin C diet. 80 kDa subunit: linear effect of S (P = 0.03); vitamin C within high S (P = 0.09); 76 kDa subunit: vitamin C versus no vitamin C (P = 0.09); vitamin C within 40% DDGS (medium and high sulfur) diets (P = 0.03); vitamin C within high S (P = 0.05); 78 kDa subunit: linear effect of sulfur (P = 0.11). Standard error of the mean: ± 3.76, 3.18, and 6.29 for 80 kDa subunit, 78 kDa subunit, and 76 kDa subunit, respectively.

Figure 2.

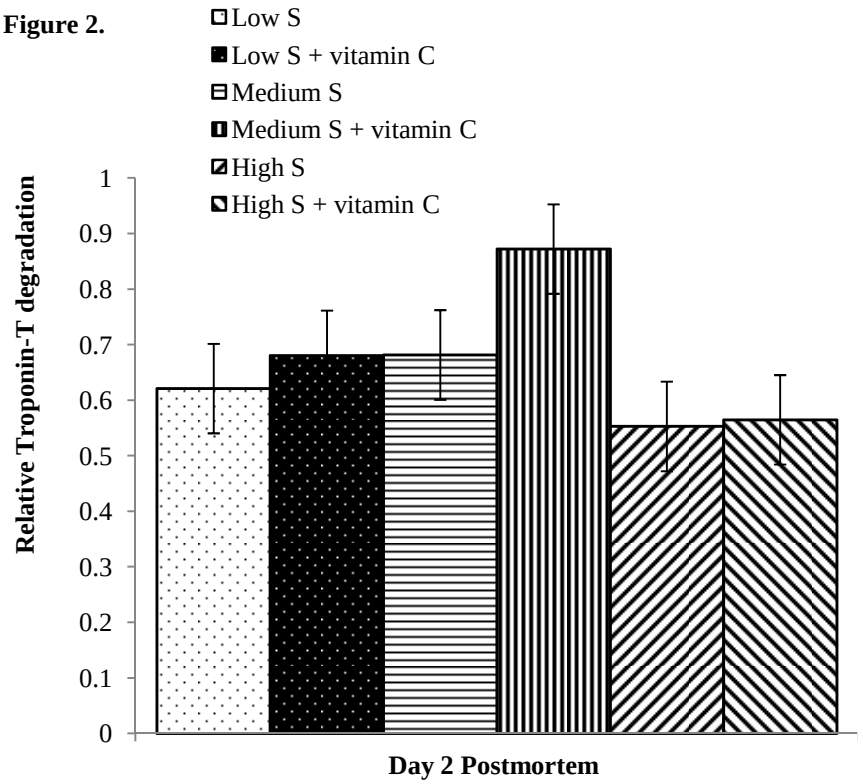

Figure 2. Effects of a rumen-protected supplemental vitamin C source on troponin-T degradation at 2-d postmortem in the *longissimus thoracis* collected from steers consuming a low sulfur (S; 0.22%), low S + 10 g·steer^{-1}·d^{-1} vitamin C, medium S (0.34%), medium S + 10 g·steer^{-1}·d^{-1} vitamin C, high S (0.55%), high S + 10 g·steer^{-1}·d^{-1} vitamin C diet. A tendency for a linear effect of S (P = 0.07). Height ratio represents the sample peak height (of the band detected via western blotting) divided by the control sample peak height; greater ratio indicates a greater degradation. Standard error of the mean: ± 0.08.

Figure 3.

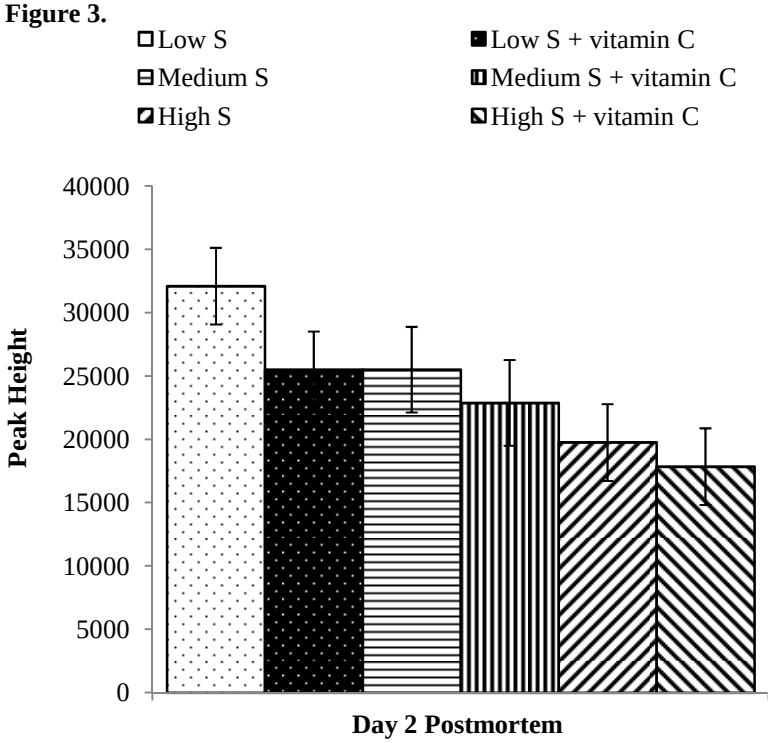

Figure 3. Effects of a rumen-protected supplemental vitamin C on myofibrillar protein carbonylation at 2-d postmortem in the *longissimus thoracis* collected from cattle consuming a low sulfur (S; 0.22%), low S + 10 g·steer^{-1}·d^{-1} vitamin C, medium S (0.34%), medium + 10 g·steer^{-1}·d^{-1} vitamin C, high S (0.55%), high S + 10 g·steer^{-1}·d^{-1} vitamin C diet. A linear effect of sulfur (P = 0.04). Peak height indicates the height of the sample band measured via western blotting, greater values indicate a greater amount of carbonylation. Standard error of the mean: ± 3144.

CHAPTER 6.

THE EFFECT OF VARYING CONCENTRATIONS OF VITAMIN C ON PERFORMANCE, BLOOD METABOLITES, AND CARCASS CHARACTERISTICS OF STEERS CONSUMING A COMMON HIGH SULFUR (0.55% S) DIET

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ABSTRACT: The objective of this study was to examine the effects of vitamin C (**VC**) supplementation for an average of 102 d prior to harvest on finishing performance and blood metabolites of steers receiving a 40% dry distillers grains plus solubles diet (0.55% S). Yearling, Angus-cross steers (n = 140) were blocked by initial BW (432 ± 25.5 kg), stratified within blocks by intramuscular fat (3.6% ± 0.30) determined by ultrasonography, and assigned to treatments (5 steers/pen, 7 pens/treatment). Treatments included: 1) no VC control (**CON**), 2) 5 g VC·steer^{-1}·d^{-1} (**5VC**), 3) 10 g VC·steer^{-1}·d^{-1} (**10VC**), and 4) 20 g VC·steer^{-1}·d^{-1} (**20VC**). Jugular blood was collected from two steers/pen prior to feeding at the beginning and end of the 102 d study, and steers were harvested by block on three separate dates (d 91, 105, and 112). Sulfur intake linearly decreased ($P = 0.01$) as VC inclusion increased (59.2, 57.7, 57.0, 54.8 ± 0.79 g S·steer^{-1}·d^{-1}, for CON, 5VC, 10VC, and 20VC, respectively). The CON cattle had greater ($P < 0.01$) DMI than the VC supplemented cattle. Inclusion of VC did not influence ADG or final BW, resulting in a tendency for a linear

increase ($P = 0.08$) in G:F as VC inclusion increased (0.150, 0.152, 0.158, 0.160 ± 0.004, for CON, 5VC, 10VC, and 20VC, respectively). Ending (2 d before harvest) plasma ascorbate showed a quadratic effect ($P < 0.05$) as a result of lesser concentrations exhibited by 5VC cattle (1,186 µg/L) compared with the CON (1,454 µg/L), 10VC (1,304 µg/L), and 20VC (1,436 µg/L; SEM ± 64.8) cattle. Ending plasma insulin concentrations of CON cattle tended ($P = 0.07$) to be less than the VC supplemented cattle. Plasma glucose and NEFA concentrations were not affected ($P \geq 0.23$) by VC inclusion. Hot carcass weight, 12th rib back fat, marbling, and quality grade were not affected ($P \geq 0.27$) by VC inclusion. Increasing VC inclusion linearly increased ($P = 0.02$) ribeye area (84.9, 86.5, 88.7, 89.1cm^2 ± 1.17, for CON, 5VC, 10VC, and 20VC, respectively), corresponding to a linear decrease ($P = 0.02$) in yield grade with increasing inclusions of VC. A tendency ($P = 0.06$) for a quadratic effect on KPH was observed, in which values generally increased from CON (2.27%), 5VC (2.37%), and 10VC (2.39%), then decreased in 20VC (2.20%). In conclusion, VC supplemented to a high S diet for an average of 102 d prior to harvest has limited effects on blood metabolites, but increased ribeye area and tended to increase feed efficiency of yearling steers.

INTRODUCTION

Due to the rise in corn prices, producers have been encouraged to seek alternative feedstuffs for feedlot cattle, such as dried distillers grains with solubles (**DDGS**). While DDGS provide an ample source of energy and protein, these products often contain increased concentrations of S, potentially limiting the inclusion rate in cattle diets. High S diets hinder

live animal performance and carcass characteristics (Zinn et al., 1997; Richter et al., 2012; Pogge and Hansen, 2013), specifically decreasing DMI, ADG, HCW, and marbling score.

The role of vitamin C (**VC**) as an antioxidant is well defined; however, the need for VC in finishing cattle diets has been examined less because of the enzymatic conversion of glucose to ascorbate (VC) in the liver of cattle, a process not found in humans (Combs, 2008). Cattle can synthesize VC, but the quantity of circulating VC decreases in fattening cattle (Takahashi et al., 1999) and across the first 90 d of cattle being fed a 0.55% S diet (Pogge and Hansen, 2013). Additionally, the supplementation of a rumen-protected VC may enhance marbling scores of cattle receiving a 0.55% S diet (Pogge and Hansen, 2013), increase ribeye area (**REA**) (Oohashi et al., 1999; Yano, 2002), and prevent the decline of circulating VC in finishing steers (Ohashi et al., 2000, Pogge and Hansen, 2013). It is still unclear what concentration of supplemental VC is needed to support live and carcass-based performance in cattle fed high S diets. The hypothesis was that an increase in marbling score would be observed in steers consuming increasing doses of VC during the later finishing period. Therefore, the objective of this study was to examine the effects of four concentrations of VC supplemented for an average of 102 d prior to harvest on cattle performance, carcass characteristics, and blood metabolites of steers receiving a 40% DDGS high S diet.

MATERIALS and METHODS

Procedures and protocols for this experiment were approved by the Iowa State University Institutional Animal Care and Use Committee (protocol number 11-11-7263-B).

Animals and Experimental Design

Yearling Angus-cross steers (n = 140) were purchased at commercial auction barns (Lamoni, IA and Denison, IA) in February 2012, and were transported to the Iowa State University Beef Nutrition Farm (Ames, IA) where steers were weighed, de-wormed with Ivomec Eprinex Pour-On for Beef and Dairy (5 mg eprinomectin/mL; Merial Animal Health, Duluth, GA), vaccinated with a modified live virus against bovine viral diarrhea types 1 and 2, infectious bovine rhinotracheitis, parainfluenza-3, and bovine respiratory syncytial virus (Pyramid3, Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO), and were identified with a unique ear tag. Steers were started on a common receiving diet for 7 d, followed by a series of four, 7-d step-up diets where corn gradually replaced corn silage and hay (roughage) and the high S DDGS gradually replaced lower S DDGS used in early step-up diets in preparation for the finishing diet (Table 1). Ultrasound measures were conducted by a certified technician on d -19 or -14 prior to start of the study, capturing REA, percent intramuscular fat of the REA, 12th rib back fat thickness (**BF**), and rump fat thickness.

At the initiation of the study (March, 2012), two consecutive day weights were taken and steers were blocked by initial BW (432 ± 25.5 kg), stratified within blocks by ultrasound-measured initial intramuscular fat (3.6% ± 0.30), and randomly assigned to one of four treatments (5 steers/pen, 7 pens/treatment), including: 1) no VC control (**CON**), 2) 5 g VC·steer^{-1}·d^{-1} (**5VC**), 3) 10 g VC·steer^{-1}·d^{-1} (**10VC**), and 4) 20 g VC·steer^{-1}·d^{-1} (**20VC**). A VC premix containing Vitashure C50 (a rumen protected ascorbate, 50% VC product; Balchem Corp., New Hampton, NY) and DDGS was used to introduce VC to the diet. The inclusion rate of the VC premix was adjusted, based on weekly DMI averages for each treatment, in order to maintain the designated VC content of 5, 10, or 20 g VC in the diet. Prior to

receiving the assigned study diets, steers were implanted with Component TE-IS (80 mg TBA and 16 mg estradiol; VetLife, Ivy Animal Health, Inc., Overland Park, KS). Single day weights were collected every 28 d and consecutive final BW were determined over the 2 d immediately prior to the harvest day. Two d before harvest, in conjunction with collection of final BW, ultrasound measures (REA, percent intramuscular fat of REA, BF, and rump fat thickness) were determined.

Four, 25-ton semi-truck loads of DDGS (POET; Jewell, IA), ranging from 0.97 to 1.04% S and 5.9 to 7.83% fat, were used in this study. Calcium sulfate (**$CaSO_4$**) was included in the diet to maintain a targeted total S concentration of 0.55% S and diets were analyzed to contain 0.55% S. The inclusion rate of $CaSO_4$ was adjusted based on the analyzed S content of the DDGS, which provided the majority of the S in the diet (Dairyland Laboratories, Arcadia, WI), and the inclusion rate of $CaSO_4$ averaged 0.60% of DM (ranging from 0.47 to 0.67% DM).

Sample Collection and Analytical Procedures

Bunk scores and the feed offered to each pen were recorded daily, and samples of individual ingredients, total mixed rations (**TMR**), and orts were weighed and sampled weekly for DM determination. Samples were dried in a forced air oven at 70°C for 48 h. Weekly pen DMI was calculated by subtracting the DM-adjusted weekly orts sample from the DM-adjusted weekly TMR. Dry matter adjustments were applied to TMR and ort samples by multiplying the percent DM of the appropriate treatment TMR or ort sample by the as-fed feed delivered to the pen and as-fed ort weight taken at the end of the week for each pen, respectively. Feed efficiency (**G:F**) was calculated every 28 d from steer weight

gain and total DMI for each 28 d interim weight period. Sulfur analysis of monthly composites of weekly TMR samples and pen orts was conducted according to the method described by Richter et al. (2012) and dietary S was calculated by multiplying the percent S by the appropriate TMR or orts samples (DM basis). Supplemental VC intake was calculated by multiplying the percent VC added to the diet (DM basis) by the daily DMI averaged from weekly DMI.

Jugular blood was collected into heparinized tubes (sodium heparin, Becton, Dickinson and Co., Franklin Lakes, NJ) for plasma analysis and serum tubes (Becton, Dickinson, and Co., Franklin Lakes, NJ) for NEFA analysis from two steers/pen prior to feeding on d 0 and 2 d prior to harvest. Blood was transported to the laboratory on ice and was centrifuged at 1,000 × *g* for 10 min at 4°C. Blood for serum collection was allowed to sit at room temperature for 1 h before centrifugation at 1,000 × *g* for 15 min. Plasma and serum were aliquoted and stored at -80°C prior to the analysis of the plasma metabolites: insulin (Mercodia, Uppsala, Sweden; catalog #10-1201-01), glucose (Wako Chemcials, North Chesterfield, VA; catalog #43990901), and ascorbate (Cayman Chemical Company, Ann Arbor, MI; catalog #700420), and serum NEFA (Wako Chemicals; catalog #999-75406-01). Plasma designated for ascorbate analysis was initially prepared and assayed as previously described by Pogge and Hansen (2013). Homeostasis model assessment (HOMA), an estimate of insulin resistance (HOMA-IR), was calculated according to Matthews et al. (1985):

$$\text{HOMA-IR: } \frac{[\text{insulin (mIU/L)}] \times [\text{Glucose (mmol/L)}]}{22.5}$$

Steers were harvested by block on three separate dates, d 91(n = 40 steers; two heaviest blocks, 637 ± 3.4 kg BW), d 105 (n = 40 steers, middle two weight blocks, 635 ± 3.2

kg BW), and d 112 (n = 60 steers, three lightest blocks, 615 ± 3.2 kg BW) when greater than 60% of steers in a pen were estimated by visual appraisal to have at least 1.27 cm of back-fat. Steers were harvested at a commercial packing facility in Denison, IA (Tyson Fresh Meats) where individual identification was maintained with each carcass following harvest. Carcasses were chilled for 24 h, after which carcasses were ribbed between the 12th and 13th rib and graded according to USDA standards by representatives of the Tri-County Carcass Futurity (Iowa State University Beef Extension, Lewis, IA), who were masked to treatment. Data collected from harvested animals [(n = 136); data were not collected from four steers due to rail-outs at the packing facility (1 on d 105 and 3 on 112)] included HCW, marbling score, BF, KPH, REA, quality grade (**QG**) and yield grade (**YG**). For carcass-adjusted performance data calculation, final BW was determined by dividing the HCW by the average dressing percentage of 61 %. A 4 % pencil shrink was applied to all live BW measures prior to calculation of ADG.

Statistical Analysis

The experimental design was a randomized complete block and the data were analyzed by ANOVA using the Mixed Procedure of SAS (SAS Institute Inc., Cary, NC). The model for the analysis of the single time point data from ultrasound measures, blood metabolites (ascorbate, glucose, insulin, NEFA, HOMA-IR), carcass-based performance, and carcass characteristics included the fixed effects of treatment and block. The GenMod procedure of SAS was used to determine treatment differences in the percentages of QG and YG within treatments. Dietary S and VC intake, DMI, ADG, and G:F data were analyzed as repeated measures and included the fixed effects of treatment, block, time of sampling, and

the interaction. Time was the repeated effect, pen was the experimental unit, and d 0 values were used as a covariate for all data analysis (n = 7/treatment). Based on the Akaike Information Corrected Criterion, autoregressive 1 was selected as the covariance structure for all repeated measures analysis. Three a priori single df contrast statements were constructed: A) no VC versus VC, B) linear effect of VC, and C) quadratic effect of VC. Significance was declared at $P \leq 0.05$ and tendencies were declared from $P = 0.06$ to 0.10.

RESULTS

Intake and Performance

Steer performance, S intake, and supplemental VC intake data are presented in Table 2. Based on repeated measures analysis across the experiment, DMI decreased ($P = 0.004$) as VC concentration increased in the diet, while G:F tended to increase ($P = 0.08$) with increasing VC inclusion to the diet throughout the finishing period. Vitamin C inclusion did not affect ADG ($P = 0.89$) or final BW ($P = 0.48$). No treatment by week ($P = 0.41$; DMI) or treatment by month interactions ($P = 0.95$; ADG and G:F) were observed. No differences due to VC supplementation ($P \geq 0.22$) were observed among final ultrasound data (data not shown).

As designed, supplemental VC intake was different ($P < 0.01$) among treatments and VC intake demonstrated a treatment by week interaction ($P < 0.001$; data not shown). This interaction is likely due to the changing rate of VC inclusion each week, as DMI did not display a similar treatment by week interaction. The VC intake interaction may be driven by a lesser ($P \leq 0.06$) VC intake during week 1 by all VC supplemented cattle. However, after week 1, VC intakes of 5VC treatment were not different ($P \geq 0.37$) throughout the remainder

of the study. The 10VC and 20VC supplemental VC intakes increased ($P \leq 0.05$) during the initial four weeks of the study, and then remained consistent throughout the remaining weeks of the study.

Based on repeated measures analysis, S intakes closely followed DMI and linearly decreased ($P = 0.01$; Table 2) with increasing concentrations of VC in the diet, and displayed a treatment by week interaction ($P < 0.001$; data not shown). The CON treatment displayed greater variation ($P \leq 0.10$) in S intakes across the initial six weeks of the study, but after week 6, S intakes were not different ($P \geq 0.19$). Within 5VC, S intakes tended to increase ($P \geq 0.09$) between the weeks 1 to 3 and 6 to 7, decreased ($P \leq 0.03$) during week 8 to 9 and 11 to 13, and remained steady throughout the rest of the trial ($P \geq 0.14$). The 10VC S intakes were not different ($P \geq 0.15$) during the initial four weeks of the study, but decreased ($P = 0.03$) between weeks 4 and 5. Sulfur intake increased ($P \leq 0.07$) during week 6 to 7, 8 to 9, and 10 to 11, while decreasing ($P \leq 0.09$) during weeks 7 to 8, 9 to 10, and 15 to 16. Within 20VC, S intakes tended to increase ($P \leq 0.10$) between weeks 2 to 4 and decrease ($P < 0.01$) between weeks 7 to 8, and remained consistent across the final weeks of the study.

Blood Metabolites

Plasma and serum metabolites are presented in Table 3. Final plasma ascorbate concentrations showed a quadratic response to supplemented VC ($P = 0.02$), in which the 5VC cattle exhibited lesser ($P \leq 0.01$) plasma ascorbate (1,186.2 µg/L ± 64.8) compared to the CON (1,454.0 µg/L) and 20VC cattle (1,436.4 µg/L), but were not different ($P = 0.21$) from the 10VC cattle (1,304.2 µg/L). The CON cattle tended to have a greater plasma VC ($P = 0.08$) and lesser plasma insulin ($P = 0.07$) concentrations compared to the VC

supplemented cattle. Increasing VC inclusion did not affect plasma glucose ($P = 0.35$) or serum NEFA ($P = 0.12$). The HOMA-IR tended ($P = 0.09$) to linearly increase as the concentration of supplemental VC in the diet increased.

Carcass Characteristics

Carcass characteristics, QG, and YG distributions across treatments are presented in Tables 4 and 5. Hot carcass weight and BF were not affected ($P \geq 0.27$) by increasing inclusion of VC. Though marbling scores and QG were not affected by dietary treatment ($P \geq 0.33$), cattle receiving supplemental VC had a lesser ($P = 0.05$) percentage of cattle grading Choice compared to CON cattle, subsequently increasing ($P = 0.02$) the percentage of the VC-supplemented steers grading Select compared to CON cattle. Analysis of KPH data revealed a tendency for a quadratic effect ($P = 0.06$), in which the 20VC tended to have less KPH compared to the 5VC ($P = 0.09$) and 10VC ($P = 0.07$; 2.37%, 2.39%, and 2.2% ± 0.07, for 5VC, 10VC, and 20VC, respectively), but was not different from CON cattle ($P = 0.48$; 2.27% ± 0.07). Increasing VC inclusion linearly increased ($P = 0.02$) REA, as the addition of 10 or 20 g of VC to a high S diet increased ($P \leq 0.03$) REA approximately 3.8 to 4.2 cm^2 compared to the CON cattle. The linear increase in REA by VC inclusion subsequently resulted in a linear decrease ($P = 0.02$) in YG, and a linear increase ($P = 0.03$) in proportion of YG1 carcasses due to supplemental VC addition to the high S diet.

DISCUSSION

Vitamin C supplementation linearly increased the REA of steers, with the greatest increases observed in the 10VC and 20VC treatments, as REA increased approximately 3.8

to 4.2 cm^2 compared to the CON cattle. Similarly, Japanese long-fed steers supplemented with 40 mg VC·kg $BW^{-1}·d^{-1}$ (rumen by-pass VC source; Yano, 2002) or 50 g VC·$steer^{-1}·d^{-1}$ of L-ascorbic acid-2-phosphate (Oohashi et al., 1999) displayed greater REA compared to un-supplemented counterparts. Results of the present study are similar to those observed by Oohashi et al. (1999), which an 8 cm^2 increase in REA was noted when cattle received supplemental VC during the latter half of the finishing period compared to the non-supplemented control and those supplemented throughout the duration of the finishing period (Oohashi et al., 1999). Conversely to the present study results, Pogge and Hansen (2013) reported no beneficial effect of supplementing 10 g VC·$steer^{-1}·d^{-1}$ during the entire finishing period to calf-fed steers fed varying concentrations of dietary S (0.22, 0.34, or 0.55%) on REA. However, the mechanisms by which VC may be altering muscle growth and REA are currently unknown.

Vitamin C supplementation did not influence marbling score in the present study; however, other researchers have observed increases in marbling score when VC was supplemented during the finishing period (Oohashi et al., 1999; Yano, 2002; Pogge and Hansen, 2013). Specifically, Yano (2002) reported that Japanese-long fed cattle supplemented with 40 mg VC·kg $BW^{-1}·d^{-1}$ (for approximately 600 d finishing period) displayed increased marbling scores from 3.25 to 5.75 (according to the Japanese grading scale), an approximate equivalent to the USDA marbling scores of modest and moderately abundant, respectively. Pogge and Hansen (2013) reported that the inclusion of VC (10 g·$steer^{-1}·d^{-1}$) to calf-fed steers (approximately 8 to 9 mo of age) consuming a 0.55% S diet for 149 d increased marbling scores from slight^{98} (Select^{+}) to small^{70} (Choice^{-}) when compared to the non-VC supplemented steers consuming the 0.55% S diet, and this addition of VC to

the 0.55% S diet recovered QG to values similar to those of steers consuming a more traditional corn-based diet (0.22% S).

The mechanism by which supplemental VC may impact marbling scores of cattle remains to be elucidated. Du et al. (2013) indicate the optimal time to nutritionally manipulate the number of adipocytes present, for the later accumulation of lipid during finishing, is from early weaning to approximately 250 d of age. In the present study, the use of yearling steers may have been the reason marbling scores were not influenced by VC supplementation, while the increase in marbling scores observed by Pogge and Hansen (2013) due to VC supplementation may be related to the use of calf-fed steers (8 to 9 mo of age).

A quadratic effect on KPH data was observed with VC supplementation, in which VC concentrations of 5 or 10 $g \cdot steer^{-1} \cdot d^{-}$ resulted in KPH values greater than the CON and 20VC treatments. These results indicate a potential threshold for VC on KPH, or perirenal adipose tissue, accumulation. An increased KPH due to VC supplementation (at 10 $g \cdot steer^{-1} \cdot d^{-1}$) was also observed by Pogge and Hansen (2013). An increase in KPH may be related to the role of glycerol-3 phosphate dehydrogenase in the conversion of glycerol-3 phosphate to glycerol for subsequent incorporation into triglycerides. Research data suggest that VC supplementation to cultured cells, specifically those extracted from the perirenal and subcutaneous adipose tissue, results in greater glycerol-3 phosphate dehydrogenase activity and enhanced triglyceride accumulation (Ono et al., 1990; Kawada et al., 1990; Torii et al., 1998; Lee et al., 2000).

Increasing the inclusion rate of VC tended to increase feed efficiency of steers, evident by a decrease in DMI of approximately 0.3 to 0.8 kg/d with no differences in ADG or

final BW among the four treatments. Pogge and Hansen (2013) reported no influence of VC supplementation on feed efficiency, but cattle were less efficient when dietary S was greater than 0.34%. However, amongst poultry and swine, increases in feed efficiency have been observed with VC supplementation, specifically in stressful situations such as heat stress and weaning (de Rodas et al., 1998; Sahin et al., 2003). While indicators of stress were not evaluated in the present study, Pogge and Hansen (2013) have reported a 0.55 % S diet may be involved in the development of oxidative stress in cattle, indicated by an increased ratio of oxidized-to-reduced glutathione in the liver. Bottje and Carstens (2009) indicated an increased ratio of oxidized-to-reduced glutathione corresponded to a less feed efficient animal. Pogge and Hansen (2013) reported the supplementation of VC (10 $g \cdot steer^{-1} \cdot d^{-}$) to the 0.55% S diet decreased the ratio of oxidized-to-reduced glutathione. Because glutathione and VC share a regenerative relationship, VC may be alleviating some of the oxidative stress occurring in steers consuming a high S diet, which may help explain the tendency to improve feed efficiency in the present study.

Currently, the NRC (1996) does not specify a daily VC requirement for cattle because of their ability to synthesize VC from glucose in the liver. Plasma ascorbate concentrations of healthy beef cattle, across all aspects of production, range from 2,400 to 4,700 µg/L (13.6 to 26.7 µM; Smith et al., 2009), and they are as low as 294 to 742 µg/L (1.66 to 4.21 µM) in finishing steers consuming a 0.55% S diet (Pogge and Hansen, 2013). Takahashi et al. (1999) noted that plasma ascorbate concentrations decreased during the fattening period and suggested that this decrease may be related to the consumption of VC as a means to control the development of oxidative stress. Similar to this response, Pogge and Hansen (2013) reported a sharp decrease in plasma ascorbate during the initial 90 d of finishing in steers

consuming a 0.55% S diet; however, when VC was included in the diet the decline in plasma ascorbate was prevented. In the present study, all cattle were fed a high S diet and no decreases in plasma ascorbate were noted during the finishing period; however, it is unknown how plasma ascorbate concentrations of steers consuming a high S diet may have compared with ascorbate concentrations in steers consuming a low S diet because such a diet was not examined in the present study.

Padilla et al. (2007) observed a linear increase in plasma ascorbate of fattening beef cows as the content of a supplemented rumen by-pass VC source increased. In the present study, it was hypothesized that plasma ascorbate concentration would be least in the non-supplemented CON cattle, and would linearly increase as the VC inclusion rate increased. However, this hypothesis was not supported by the experimental data. Despite supplementation of a rumen-protected VC source to three of the four treatment groups, the un-supplemented CON cattle exhibited the greatest plasma ascorbate concentration. While the reason for the unexpected plasma ascorbate differences is unknown, it may be related to individual animal variation or may be the result of changes in endogenous production of VC due to VC supplementation. Tsao and Young (1990) reported exogenous supplementation of VC to mice decreased the production of VC by the liver, and authors suggested the production of endogenous VC might be directly related to the concentration of VC in the portal blood. Interestingly, plasma ascorbate concentrations in the present study were markedly greater than those measured by Pogge and Hansen, 2013. The results of the current study and those of Pogge and Hansen (2013) suggest a potential for a threshold for circulating ascorbate, which may be influenced by the age of the cattle at the time of exposure to a high S finishing diet. The differing responses of plasma ascorbate

concentrations and marbling scores between the current study and Pogge and Hansen (2013) may suggest a certain plasma ascorbate concentration is necessary to support marbling.

The hypothesis was that the supplementation of VC to cattle diets may provide a sparing mechanism for glucose to be used for other body functions rather than the synthesis of VC. However, glucose and NEFA concentrations were not different between treatments. Alternately, Oohashi et al. (1999) reported a lesser concentration of circulating glucose in cattle supplemented with 50 $g \cdot steer^{-1} \cdot d^{-1}$ of L-ascorbic acid-2-phosphate for the entire finishing period compared to those supplemented for a portion (early or later) or not receiving supplemental VC at all during the finishing period. Similar to the findings of the present study, these authors also reported no difference in NEFA concentration among treatments (Ooshahi et al., 1999). Interestingly, the supplementation of VC in the present study tended to increase plasma insulin concentrations by approximately 0.37 to 0.57µg/L compared to CON steers, which corresponds with an increased HOMA-IR indicating more insulin resistance in VC supplemented steers. No additional information is available concerning the influence of VC on plasma insulin concentrations in feedlot cattle.

In conclusion, the results of this study suggest that the supplementation of 5 to 20 g $VC \cdot steer^{-1} \cdot d^{-}$, during the later finishing period (91 and 112 d prior to harvest), to yearling steers consuming a high S diet increases REA and may potentially improve feed efficiency, while having limited effects on blood metabolites and only a tendency to affect other carcass characteristics. However, further research is required to determine the exact mechanism by which VC supplementation is altering feed efficiency and supporting muscle development.

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Table 1. Ingredient composition of finishing diets (% DM basis)

Ingredient	Common Diet[1]
Corn	45.0
Corn dried distiller's grains[2,3]	40.0
Chopped hay	6.5
Corn silage	5.5
Limestone	1.4
Salt	0.3
Vitamin A premix[4]	0.1
Trace mineral premix[5]	0.035
Rumensin90[6]	0.01
Calcium sulfate[7]	0.60
S[8], %	0.54
Calculated composition[9]	
% Lipid	5.08
Vitamin E, IU·kg^{-1} diet DM	304.3

[1]Treatments: CON: control; 5VC: 5 g vitamin C·steer^{-1}·d^{-1}; 10VC: 10 g vitamin C·steer^{-1}·d^{-1}; 20VC: 20 g vitamin C·steer^{-1}·d^{-1}.

[2]Vitashure C (donated by Balchem Corp., New Hampton, NY) replaced distillers grains plus solubles (DDGS), by 0.11 to 0.43% diet DM, to achieve the target level of vitamin C per steer per day.

[3]Four loads of DDGS (POET; Jewell, IA) were used during the trial, S concentrations were: 0.97, 1.04, 0.96, and 0.96% and fat content: 7.83, 6.91, 5.90, and 6.25%.

[4]Vitamin A premix contained 4,400,000 IU·kg^{-1}.

[5]Provided per kg of diet: 30 mg Zn as $ZnSO_4$; 20 mg Mn as $MnSO_4$; 0.5 mg I as $Ca(IO_3)_2(H_2O)$; 0.1 mg Se as Na_2SeO_3; 10 mg Cu as $CuSO_4$; and 0.1 mg Co as $CoCO_3$

[6]Provided at 27g/t diet (donated by Elanco Animal Health).

[7]Calcium sulfate was included at an average of 0.60% diet DM (range of 0.47 to 0.67%), at the expense of DDGS, to targeted S content in the diet.

[8]S content for the four treatments are repeated measures least squares mean averages throughout the entire study.

[9]Lipid content was calculated from the analyzed lipid content of individual ingredients and vitamin E concentrations were calculated based on NRC values for each ingredient.

Table 2. The effect of varying concentrations of supplemental vitamin C (VC) on dry matter intake, gain, and efficiency of steers consuming a common high S (0.55% S) diet

	CON[1]	5VC[1]	10VC[1]	20VC[1]	SEM	Contrast Statements[2] *P* values		
Live performance						CON vs. VC	Linear VC	Quad VC
Initial weight[3], kg	432	433	432	432	0.4	0.40	0.61	0.27
Final weight[3], kg	604	599	606	599	3.6	0.56	0.48	0.55
DMI[4,5], kg/d	11.40	10.84	11.09	10.61	0.148	0.004	0.004	0.65
ADG[4,6], kg/d	1.67	1.62	1.71	1.64	0.059	0.84	0.89	0.71
G:F[4,6]	0.150	0.152	0.158	0.160	0.0042	0.16	0.08	0.61
S intakes[7], g/d	59.2	57.7	57.0	54.8	0.79	0.01	0.01	0.82
Carcass-adjusted performance[8]								
Final weight, kg	603	600	606	598	3.5	0.62	0.42	0.44
ADG, kg/d	1.49	1.45	1.52	1.44	0.03	0.63	0.45	0.44
G:F	0.133	0.136	0.139	0.140	0.0033	0.19	0.14	0.61

[1]Treatments: CON: control; 5VC: 5 g vitamin C·steer^{-1}·d^{-1}; 10VC: 10 g vitamin C·steer^{-1}·d^{-1}; 20VC: 20 g vitamin C·steer^{-1}·d^{-1}.

[2]Contrast Statements: CON vs. VC = no vitamin C vs. vitamin C; Linear VC = linear effect of vitamin C; Quad VC = quadratic effect of vitamin C.

[3]A 4% pencil shrunk was applied to weights.

[4]Dry matter intake, S intake, ADG, and G:F were analyzed as repeated measures.

[5]Week $P < 0.001$; Treatment by week $P > 0.41$.

[6]Month $P < 0.001$; Treatment by month $P \geq 0.95$.

[7]Week $P < 0.001$; Treatment by week $P < 0.001$.

[8]Carcass-adjusted performance values are based on final BW calculated from HCW divided by the average dressing percent of 61% for all treatments; a 4% pencil shrink was applied to initial weights prior; ADG and G:F were calculated over the total days on feed.

Table 3. The effect of varying concentrations of supplemental vitamin C (VC) on blood metabolites of steers consuming a common high S (0.55% S) diet

	CON[1]	5VC[1]	10VC[1]	20VC[1]	SEM	Contrast Statements[2] *P* values		
						CON vs. VC	Linear VC	Quad VC
Plasma metabolites								
Ascorbate[3], µg/L	1,454.0	1,186.2	1,304.2	1,436.4	64.8	0.08	0.53	0.02
Insulin[3], µg/L	1.28	1.65	1.85	1.69	0.19	0.07	0.23	0.11
Glucose[3], mg/dL	99.27	98.43	96.08	103.64	3.31	0.98	0.35	0.23
HOMA-IR[4]	11.09	13.86	15.14	18.74	2.76	0.17	0.09	0.88
Serum NEFA[3], µEq/L	189.69	175.18	170.82	228.58	19.6	0.93	0.12	0.11

[1]Treatments: CON: control; 5VC: 5 g vitamin C·steer^{-1}·d^{-1}; 10VC: 10 g vitamin C·steer^{-1}·d^{-1}; 20VC: 20 g vitamin C·steer^{-1}·d^{-1}.

[2]Contrast Statements: CON vs. VC = no vitamin C vs. vitamin C; Linear VC = linear effect of vitamin C; Quad VC = quadratic effect of vitamin C.

[3]Jugular blood was drawn prior to feeding 2 d before harvest.

[4]Homeostasis model assessment, insulin resistance.

Table 4. The effect of varying concentrations of supplemental vitamin C (VC) on carcass characteristics[1] of steers consuming a common high S (0.55% S) diet

	CON[2]	5VC[2]	10VC[2]	20VC[2]	SEM	Contrast Statements[3] *P* values		
						CON vs. VC	Linear VC	Quad VC
HCW, kg	387	383	388	383	2.29	0.44	0.40	0.57
Dressing percent	61.4	61.4	61.5	61.4	0.24	0.91	0.82	0.88
12th rib BF, cm	1.33	1.30	1.35	1.23	0.06	0.59	0.27	0.47
KPH, %	2.27	2.37	2.39	2.20	0.07	0.53	0.32	0.06
REA[4], cm^2	84.9	86.5	88.7	89.1	1.17	0.03	0.02	0.29
Yield grade	3.28	3.17	3.15	2.93	0.10	0.09	0.02	0.86
Marbling score[5]	478	465	461	456	14.83	0.32	0.34	0.66
Quality grade[6]	3.28	3.0	3.0	3.0	0.16	0.16	0.33	0.35

[1]Cattle were harvested on 3 separate days by blocks: d 91 (n = 40); d 105 (n = 39); d 112 (n = 57)

[2]Treatments: CON: control; 5VC: 5 g vitamin C·steer^{-1}·d^{-1}; 10VC: 10 g vitamin C·steer^{-1}·d^{-1}; 20VC: 20 g vitamin C·steer^{-1}·d^{-1}

[3]Contrast Statements: CON vs. VC = no vitamin C vs. vitamin C; Linear VC = linear effect of vitamin C; Quad VC = quadratic effect of vitamin C

[4]Ribeye area

[5]Marbling scores: slight: 300, small: 400, modest: 500

[6]Quality grade: 2: Select$^+$, 3: Choice$^-$, 4: Choice

Table 5. The effect of varying concentrations of supplemental vitamin C (VC) on the distribution of quality and yield grades of steers consuming a common high S (0.55% S) diet

	CON[1]	5VC[1]	10VC[1]	20VC[1]	Contrast Statements[2] *P* values		
					CON vs. VC	Linear VC	Quad VC
Quality grade[3], %							
Prime	3	0	3	3	0.46	0.72	0.25
Choice	89	71	74	71	0.05	0.09	0.14
Select	9	29	23	26	0.02	0.08	0.09
Standard	0	0	0	0	1.0	1.0	1.0
Yield grade[3], %							
1	0	0	6	11	1.0	0.03	0.30
2	32	49	37	31	0.49	0.60	0.29
3	62	43	43	54	0.12	0.76	0.07
4 and 5	6	9	14	3	0.76	0.49	0.10

[1]Treatments: CON: control; 5VC: 5 g vitamin C·steer^{-1}·d^{-1}; 10VC: 10 g vitamin C·steer^{-1}·d^{-1}; 20VC: 20 g vitamin C·steer^{-1}·d^{-1}.

[2]Contrast Statements: CON vs. VC = no vitamin C vs. vitamin C; Linear VC = linear effect of vitamin C; Quad VC = quadratic effect of vitamin C.

[3]USDA quality and yield grades are based on percentages within treatments.

CHAPTER 7.

INFLUENCE OF SUPPLEMENTING VITAMIN C TO YEARLING STEERS FED A HIGH SULFUR DIET DURING THE FINISHING PERIOD ON MEAT COLOR, TENDERNESS AND PROTEIN DEGRADATION, AND FATTY ACID PROFILE OF THE *LONGISSIMUS* MUSCLE

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ABSTRACT: The objective was to determine the influence of vitamin C (**VC**) supplemented for approximately 102 d during the finishing period on color, tenderness, and fatty acid profile of *longissimus thoracis* (**LT;** n = 136) from steers fed a 0.55% sulfur diet. Treatments included 4 supplemental VC concentrations: 1) 0 (**CON**), 2) 5 (**5VC**), 3) 10 (**10VC**), or 4) 20 (**20VC**) $gVC \cdot h^{-1} \cdot d^{-1}$ in a common diet. Increasing supplemental VC decreased ($P < 0.01$) L*, but increased ($P < 0.01$) vitamin E and tended to increase ($P \leq 0.07$) calcium and iron content of steaks. No VC ($P \geq 0.25$) effect was noted for WBSF, calpain-1 autolysis, troponin T degradation, or most fatty acid profiles. A quadratic effect ($P \leq 0.03$) was observed for cholesterol and CLA content of LT. Under the conditions of our study, supplementing VC to steers fed a 0.55% sulfur diet late in the finishing period did not influence color or tenderness, but increased the vitamin E content.

INTRODUCTION

In cattle diets inclusion of dried distillers grains plus solubles (**DDGS**) is often associated with greater dietary sulfur (**S**) concentrations, as the S content of DDGS can range from 0.3 to greater than 1% S (Kim, Zhang, & Stein, 2012). However, due to economic incentive, greater amounts of DDGS are being incorporated into cattle diets, thus exposing cattle across the United States to greater dietary concentrations of S than have been traditionally fed. While many studies have focused on the negative effects of high S diets on feedlot cattle performance, health, and carcass traits (Buckner, Erickson, Mader, Colgan, & Karges, 2007; Pogge & Hansen, 2013*a*; Richter, Drewnoski, & Hansen, 2012; Uwituze et al., 2011; Zinn et al., 1997), very little research data are available specifically addressing the possible residual effects of a high S diet on meat quality attributes.

Postmortem protein oxidation in meat has the potential to affect fresh beef quality (Huff-Lonergan, Zhang, & Lonergan, 2010). Rowe, Maddock, Lonergan, & Huff-Lonergan (2004) observed an oxidative environment, induced by irradiation of beef steaks, hindered the ability of calpain-1 to exert proteolytic activity, thus limiting protein degradation and tenderness of steaks. That same investigation demonstrated that supplementation of cattle with vitamin E (**VE**) resulted in lower calpastatin activity in muscle at 1 d postmortem. Finally, high oxygen environments create protein polymerization and meat toughening in beef (Kim, Huff-Lonergan, Sebranek, & Lonergan, 2010).

Sulfur has been shown to be a contributing factor in the development of oxidative stress (Pogge & Hansen, 2013*a*; Truong, Eghbal, Hindmarsh, Roth, & O'Brien, 2006). In addition to contributing greater concentrations of S, DDGS have traditionally been an excellent source of dietary fat and have been previously reported to increase the

concentration of polyunsaturated fatty acids (**PUFA**) deposited in the muscle and adipose tissue of beef cattle (Aldai et al. 2010; Dugan et al., 2010; Koger et al., 2010; Mello Jr. et al., 2012; Pogge, Lonergan, & Hansen, 2013; Vander Pol, Luebbe, Crawford, Erickson, & Klopfenstein, 2009). However, greater concentrations of PUFA in postmortem muscle, especially in an oxidative environment, can decrease the stability of meat lipid and color resulting in the production of off-flavors and meat surface discoloration (Renerre, Dumont, & Gatellier, 1996; Wood et al., 2004).

Due to the negative alterations an oxidative environment imposes on meat, the use of exogenous antioxidants, such as vitamins C (**VC**) and E, have been added to animal diets or incorporated during meat processing as a means to maintain the reducing environment and prolong shelf life (Greene & Price, 1975; Mitsumoto, Cassens, Schaefer, & Scheller, 1991; Rowe et al., 2004). Our hypothesis was that increasing the concentration of supplemental VC to steers consuming a 0.55% S diet would increase the muscle concentrations of the antioxidants VC and VE to prolong the reducing environment in muscle, thereby increasing color and lipid stability and protein degradation. To evaluate this hypothesis, a study was designed to determine the effects of supplementing a rumen-protected VC for approximately 102 d prior to harvest on meat color, tenderness, and fatty acid profile of the *longissimus thoracis* (**LT**).

MATERIALS and METHODS

Procedures and protocols for the cattle experiment were approved by the Iowa State University Institutional Animal Care and Use Committee, protocol number 11-11-7263-B.

Animals and Experimental Design

Angus-cross yearling steers (n = 140) were purchased at a commercial auction barn and were transported in February (2012), to the Iowa State University Beef Nutrition Farm. At the initiation of the study (March, 2012), steers were blocked by initial body weight (432 ± 25.5 kg), stratified within blocks by ultrasound-measured initial intramuscular fat (3.6% ± 0.30), and were randomly assigned to one of four treatments (5 steers/pen, 7 pens/treatment), including: 1) high S (0.55%) 40% DDGS, no VC control (CON), 2) CON high S diet + 5 g $VC \cdot h^{-1} \cdot d^{-1}$ (5VC), 3) CON high S diet + 10 g $VC \cdot h^{-1} \cdot d^{-1}$ (10VC), and 4) CON high S diet + 20 g $VC \cdot h^{-1} \cdot d^{-1}$ (20VC; Table 1). The VC product (Vitashure C50) is a rumen protected 50% VC product (Balchem Corp., New Hampton, NY). Over the course of the trial steers receiving supplemental VC consumed 0, 5.1, 10.1, and 20.2 $g \cdot h^{-1} \cdot d^{-1}$, for CON, 5VC, 10VC, and 20VC respectfully, and the daily S intake was 59.2, 57.7, 57.0, 54.8 ± 0.79 g $S \cdot h^{-1} \cdot d^{-1}$, for CON, 5VC, 10VC, and 20VC respectfully (Pogge & Hansen, 2013*b*). More detailed information regarding the live animal portion of this study has been previously reported (Pogge & Hansen, 2013*b*).

Steers were slaughtered by block on three separate dates, d 91(40 steers; two heaviest blocks), d 105 (40 steers, middle two blocks), and d 112 (60 steers, three lightest blocks) when greater than 60% of steers in a pen were estimated by visual appraisal to have at least 1.27 cm of back fat. At slaughter the steers averaged 602 ± 3.6 kg BW. Steers were slaughtered at a commercial packing facility in Denison, IA (Tyson Fresh Meats) where individual identification was maintained with each carcass following harvest. Carcasses (n = 136) were chilled for 24 h, after which carcasses were ribbed between the 12^{th} and 13^{th} rib and graded according to USDA standards by representatives of the Tri-County Carcass

Futurity (Iowa State University Beef Extension, Lewis, IA), who were masked to treatment. Data were not collected from four carcasses due to rail-outs [d 91 (n = 0), d 105 (n = 1), and d 112 (n = 3)]. Note that carcass data have been previously reported in Pogge and Hansen (2013*b*). Briefly, no effects of supplemental VC were noted on hot carcass weight or marbling score; however, ribeye area linearly increased and yield grade linearly decreased with increasing VC supplementation.

Following a 24 h chill, approximately a 7.62 cm thick rib-section, cut across the length and width of the LT muscle, and the 12^{th} rib, was removed from the right side of each carcass (n =136). Rib-sections collected from each steer were placed in plastic bags with an identification tag and transported on ice back to the Iowa State University Meat Laboratory. In the Meat Laboratory, samples were de-boned and divided into three separate steaks (across the length and width of the LT). One, 1.27 cm thick steak from each carcass was held under retail conditions for 7 d for the analysis of color and shear force determination (d 7). The remaining steak sample (approximately 5.1 cm) from each rib-section was trimmed of excess fat, divided in two, vacuum packaged along with an identification card, and stored at -20°C until further analysis. Prior to analysis samples were powdered in a Waring blender (Torrington, CT) using liquid N_2 to ensure a homogenized sample.

Color Analysis

Steaks (1.27 cm thick; n = 136) designated for retail simulated display were placed on a styrofoam tray with an identification tag and wrapped with an oxygen permeable overwrap. Steaks were refrigerated for 7 d at 2°C under retail simulated lighting (7.0 lux of Deluxe Cool White fluorescent light; constant lighting 24 h/day; Osram Sylvania, Danveres, MS).

Instrumental surface color analysis was conducted initially on d 1 (approximately 30 h postmortem; after allowing steaks to bloom for 2 h under the retail lighting), d 2, 3, and 7 (at the end of display). Color analysis was determined using a Hunter LabScan XE Spectrophotometer; Hunter Associates Laboratory, Inc., Reston, VA). Illuminant D65 was used, and the instrument had a 10° observer and a port diameter of 12.7 mm. Three separate areas across the surface of the steak were measured for L*, a*, and b* values, and the collected values for each measure were averaged for each sample for statistical analysis. Hue angle (degree of true redness, 0° = true red; 90° = true yellow) and saturation index (greater values indicating more vivid color) of each steak was determined according to the equation previously reported by Gardner, Huff-Lonergan, Rowe, Schultz-Kaster, & Lonergan (2006).

Warner-Bratzler Shear Force Determination

The Warner-Bratzler shear force (**WBSF**) method was employed to determine the tenderness of steaks. At the conclusion of the 7 d retail display period, steaks were removed from the oxygen permeable wrap and placed in vacuum packaging with its corresponding identification card, and stored at -20°C until WBSF analysis. Steaks were analyzed by harvest date, to ensure equal time (60 d) spent in the freezer. Samples were thawed at 2°C for 24 h prior to cooking. Steaks were cooked on clam-shell grills to an internal temperature of 71°C, which was individually monitored on each steak using thermocouples. Steaks were cooled to room temperature prior to the removal of six core samples (approximately 0.63 cm, oriented parallel with the grain of the muscle fibers) from each steak. The Instron Universal Testing Machine (Model 5566, Instron Corporation, Norwood, MA) was used to determine the peak force (N of shear force) necessary to shear, perpendicularly, through the 0.63 cm

cores 10kN load cell, 200 mm/min). The six determinations per steak were averaged into a single value for statistical analysis.

Western Blot Analysis

Whole muscle protein extraction and protein concentration were determined according to methods previously described by Lonergan, Huff-Lonergan, Wiegand, & Kriese-Anderson (2001) and Lowry, Rosebrough, Farr, & Randall (1951), respectively. Myofibrillar protein extraction, gel composition, and western blotting for d 2 and d 7 troponin T (n = 84, 3/pen) and d 2 calpain-1 (n = 84, 3/pen) were conducted according to a modified method previously reported by Rowe et al. (2004). The modification to the procedure described by Rowe et al. (2004) is in relation to differences in antibody dilutions. The primary antibody for troponin T measurements, monocolonal anti-troponin-T (T6277; clone JLT-12; mouse IgG1 isotype; Sigma-Aldrich, St. Louis, MO) was diluted 1:10,000, and the primary antibody for calpain-1 analysis was monoclonal anti-calpain-1 (MA3-940; Affinity Bioreagents, Inc., Golden, CO) diluted 1:10,000. A common secondary antibody, goat anti-mouse-HRP (A-2554; Sigma-Aldrich), was diluted 1:10,000 and 1:5,000 for troponin-T and calpain-1, respectively. ECL Prime Western Blotting Detection System (GE Healthcare, Piscataway, NJ) was used to develop blots, and images were captured using a ChemiImager 5500 (Alpha Innotech, San Leandro, CA) and Alpha Ease FC software (v 3.03 Alpha Innotech). The bands quantified using densitometry included the 30 kDa troponin T degradation product, and the 80, 78, and 76 kDa autolysis bands of calpain-1.

Mineral Concentrations

Concentrations of S, calcium (**Ca**), and iron (**Fe**; n = 84, 3/pen) of the LT were determined utilizing inductively coupled plasma spectrometry (Optima 7000 DV, Perkin-Elmer, Waltham, MA), according to a modified method previously described by Pogge & Hansen (2013*a*). Briefly, approximately 1 g of homogenized LT (wet tissue) was digested in 9 mL of trace metal grade nitric acid through closed vessel digestion in a Mars Xpress microwave (CEM Corporation, Matthew, NC), and brought to a final volume of 50 mL with deionized water. A bovine liver standard (National Institute of Standards and Technology, Gaithersburg, MA) and an internal standard, Yttrium (Inorganic Ventures, Christiansburg, VA), were included in each session to verify instrument accuracy and account for any variation in sample introduction.

Muscle Content of Collagen, Vitamins C and E, and Cholesterol

Collagen content (n = 84, 3/pen) of d 2 LT was determined by the measurement of hydroxyproline. Steak samples were prepared by homogenizing 0.1 g of powdered steak sample with 1 mL of distilled water. A 1:1 ratio of sample homogenate and ~12 N HCl was placed in a pressure tight, Teflon-capped glass vial and hydrolyzed for 3 h at 120°C. Ten µL of hydrolyzed sample was pipetted into a 96 well plate and evaporated to dryness under vacuum. The assay reaction was conducted according to manufacturer's instruction (Bio-Vision Inc., catalog #K555-100, Milpitas, CA). A conversion factor of 7.52 was applied to determine collagen content from hydroxyproline content (Cross, Carpenter, & Smith, 1973).

Ascorbic acid content of the LT (n = 84; 3/pen) was determined utilizing high performance liquid chromatography (**HPLC**). A 5 g sample of d 2 LT was accurately

weighed into a test tube and homogenized with 15 mL of a solution containing 4.5% metaphosphoric acid, 0.3 M trichloroacetic acid and 0.05% EDTA in a polytron (Type PT 10/35, Brinkman, Westbury, NY) for 1 min at full speed. The sample was centrifuged at 3,000 × *g* for 30 min, and the supernatant collected and filtered through a 0.45 mm Millipore (Bedford, MD) filter. One mL of filtrate was transferred to a test tube containing 0.2 mL of dithiothreitol (20 mg/mL), and allowed to stand in darkness for 2 h to convert all the VC in the sample to the reduced form. An ascorbic acid standard curve was prepared using an ascorbic acid stock solution (1 g/100 mL) in 3% (w/v) metaphosphoric acid. An HPLC unit (HP 1100 system, Hewlett-Packard Co., Wilmington, DE) was used to determine VC in the sample. The column used was a Zorbax SB-C8 (4.6 × 150 mm, 5 µm) with a guard-column of the same material. Two solvents: A): 50 mM monosodium phosphate (pH = 2.5)/methanol (90/10) and B): 50 mM monosodium phosphate (pH = 2.5)/methanol (10/90) were used as eluting solvents. Elution was carried in gradient: 0-70% B in 10 min at 25°C and the flow rate was 0.5 mL/min. The eluate was monitored at 245 nm and the amount of VC in the sample was calculated using the standard curve.

Vitamin E and cholesterol content (n = 84; 3/pen) of the d 2 LT was determined using gas chromatography (GC; model 6890, Hewlett-Packard Co., Wilmington, DE). Two g of meat was accurately weight into a test tube with a cap and 10 mL of freshly prepared saponification reagent (ethanol and 33% aqueous potassium hydroxide, 94:6 ratio), 0.5 mL of 20% ascorbic acid solution, and 50 µL of 5α-cholestane solution (internal standard, 1 mg/ml in hexane) were added to each tube. Samples were homogenized using a polytron (type PT 10/35, Brinkman, Westbury, NY) for 5 s at full speed, capped, and incubated for 1 h at 50°C. After cooling at room temperature for 10 min, 5 mL deionized distilled water and 5 mL of

hexane were added. The tubes were capped tightly and mixed by vigorous shaking. After a 15 h separation phase, 1 mL of the hexane layer containing unsaponifiables was carefully transferred to GC vile and dried under nitrogen flow. To the dried sample, 200 µL of pyridine and 100 µL of Sylon BFT (99% N, O-bis(trimethylsilyl)trifluoroacetamide + 1% trimethylchlorosilane; Supleco, Bellefonte, PA) were added, tightly capped, and derivitized in a 50°C water bath for 1 h. Analysis of tocopherol and cholesterol was performed using a Hewlett-Packard 6890 model equipped with an autosampler and an FID detector. A combined column (Hewlett-Packard-5 column, 30 m × 0.25 mm i.d., 0.25 µm nominal and an Hewlett-Packard-35 column, 15 m × 0.25 mm i.d., 0.25 µm nominal) was used to improve separation between cholesterol and tocopherol. A splitless inlet was used to inject samples (1 µL) into a capillary column and ramped to oven temperature was used (260°C held for 2 min, increased to 290°C at 3°C per min, and then held at 290°C for 10 min). Column flow was maintained at 1.1 mL/min and the detector temperature was set at 280°C. The inlet and detector temperatures were 290°C and 300°C, respectively. Helium was the carrier gas at a constant flow of 1.1 mL/min. Detector (FID) air, hydrogen, and helium flows were set at 136 mL/min, 35 mL/min, and 45 mL/min, respectively. Cholesterol and tocopherol were confirmed using the retention times of standards. The area of each peak was integrated using the ChemStation software (Hewlett-Packard Co., Wilmington, DE) and quantities of both cholesterol and tocopherol were calculated using an internal standard.

Fatty Acid Analysis

Lipid extraction and determination from d 2 LT (n = 84; 3 steaks/pen) was conducted according to the methods previously described by Christie (1972), Folch, Lees, & Stanley

(1957), Richter et al., (2012), and Kramer, Hernandez, Cruz-Hernandez, Kraft, & Dugan (2008). Fatty acids were extracted from 2 g of powdered muscle tissue (wet), and the total lipid extracted from each steak was calculated as a percentage of the original 2 g of wet tissue. Lipid was extracted according to the acetyl chloride/methanol method described by Christie (1972). Extracted lipid was standardized to achieve 40 mg esterified lipid, which was measured into an airtight Teflon-capped tube, and each sample was evaporated under nitrogen gas. Following evaporation 1 mL of methanol and 100 µL of acetyl chloride were added to each tube, and samples were purged with N gas before capping. Using a heating block, samples were heated to 80°C for 1 h and vortexed every 20 min. After heating for 1 h, samples were cooled to room temperature. To each tube 5 mL of 4% potassium carbonate and 2 mL of hexane were added, samples were purged with nitrogen gas, vortexed, and centrifuged at 1700 rpm for 10 min. The esterified lipid layer (top layer) was removed and transferred to a GC vile, purged with nitrogen gas to limit fatty acid oxidation, and stored at -20°C until analysis. Samples were analyzed using a GC (model 3900; Varian Analytical Instruments, Walnut Creek, CA), using a 100-m × 0.25-mm × 0.2-µm fused silica capillary column (Supelco, Bellefonte, PA), and flame ion detector. Helium was the carrier gas. A temperature-programmed procedure was used for the analysis. The initial column temperature was 120°C for 1 min; temperature was then increased 10°C/min until the column temperature reached 175°C. This temperature was maintained for 10 min, after which it was increased by 5°C/min until the column temperature reached 210°C and 230°C, where each of these temperatures were maintained for 5 min before increasing to the next. The peak temperature was 240°C which was maintained for 5 min. The injector and detector temperature remained constant at 220 and 250°C, respectfully, throughout. One µL of fatty

acid methyl esters (**FAME**) sample was injected into the GC with split ratio 99. The fatty acid composition was calculated using the sum of all peaks, and is reported on the basis of mg of fatty acids/100 g fresh meat. No internal standard was used, but FAME standard gas-liquid chromatography (**GLC**) 68D, GLC 79, GLC 81, and GLC 461 (Nu-Chek Prep, Inc., Elysian, MN) were used to determine peak identification and quantification (Kramer et al., 2008). The percent saturated fatty acids (**SFA**), monounsaturated fatty acids (**MUFA**), polyunsaturated fatty acids (**PUFA**), omega 3 fatty acids, and omega 6 fatty acids, and the ratio of PUFA-to-SFA and omega 3-to-6 fatty acids were calculated. Atherogenic index (**AI)** was calculated according to the equation described by Ulbricht & Southgate (1991):

$$\frac{((C12:0) + (4 \times C14:0) + (C16:0))}{\%\ MUFA + \%\ PUFA}$$

An index for Δ-9-desaturase of C16 and C18 was calculated according to the equation reported by Malau-Aduli, Siebert, Bottema, & Pitchford (1998):

Δ-9-desaturase (16) index: C16:1n7 / (C16:0 + C16:1n7) *100

Δ-9-desaturase (18) index: C18:1c9 / (C18:0 + C18:1c9) *100

Statistical Analysis

Data were analyzed by ANOVA as a complete randomized block design using PROC MIXED of SAS version 9.2 (SAS Institute Inc., Cary, NC). The model for the analyses of single time point data from WBSF, fatty acid analysis, Western blot analysis (troponin T and calpain-1 autolysis), mineral concentration (S, Ca, and Fe), and nutrient content (VC, VE, cholesterol, and collagen) included the fixed effects of treatment and block. Color data were analyzed as repeated measures and included the fixed effects of treatment, block, day of

sampling, and the interactions. Day was the repeated effect, and pen was used as the experimental unit for all data analysis (n = 7/treatment). Autoregressive 1 was selected as the covariance structure for all repeated measures analysis, based on the lowest Akaike Information Corrected Criterion. Three a priori single degree of freedom contrast statements were constructed: A) no VC versus VC, B) linear effect of VC, and C) quadratic effect of VC. Procedure CORR of SAS was used to generate Pearson correlation coefficients to determine the relationship between color measures, WBSF, troponin T degradation (d 2 and 7), calpain-1 autolysis, VE, VC, and cholesterol content, and Ca and Fe concentration. For all measures significance was declared at $P \leq 0.05$, P values >0.05 and ≤ 0.10 were considered tendencies.

RESULTS

Color Analysis, Warner-Bratzler Shear Force, and Mineral Concentrations

Color analysis, Warner-Bratzler shear force, and mineral data are reported in Tables 2 and 3. Regardless of VC concentration in the diet, steaks supplemented with VC had decreased L* (lightness) values ($P = 0.005$) compared to CON. This difference resulted in a linear effect of VC on L* values ($P = 0.03$), as the VC supplemented treatments were not different ($P \geq 0.46$) from each other. Vitamin C did not influence ($P \geq 0.56$) a* (redness) or b* (yellowness) values of steaks and no treatment by day effects ($P \geq 0.58$) were observed for L* (lightness), a* (redness), or b* (yellowness) measurements. Warner-Bratzler shear force values were not influenced by treatment ($P \geq 0.43$). The content of Fe in the meat tended to increase ($P = 0.06$) as VC supplementation increased, which is primarily related to the differences ($P = 0.03$) between the CON and 20VC treatments. The concentration of Ca

in the steaks tended to respond quadratically ($P = 0.07$) to dietary VC supplementation, which is primarily being driven by the lesser ($P = 0.02$) concentration of Ca in 5VC compared to the CON and 20VC treatments. The S content of the muscle was not influenced ($P = 0.68$) by VC supplementation.

Nutrient Content of the Longissimus Thoracis Muscle

Collagen, VC, VE, and cholesterol content of the LT muscle are presented in Table 3. Vitamin C supplementation did not influence ($P \geq 0.26$) collagen content or VC content of the LT. However, the supplementation of VC, regardless of dose, increased ($P = 0.001$) the concentration of VE present in the muscle compared to CON. A quadratic effect ($P = 0.03$) was observed in VE concentration in the meat, which is primarily related to the tendency for greater ($P \leq 0.09$) concentration of VE of the 5VC compared to CON, 10VC, and 20VC. No differences ($P = 0.47$) in VE concentration were detected between 10VC and 20VC treatments. The cholesterol concentration of the muscle tended to be lesser ($P = 0.09$) in CON cattle compared to VC supplemented treatments. A quadratic effect ($P = 0.008$) was noted in cholesterol concentration in meat, which is primarily being driven by the greater ($P \leq 0.03$) cholesterol content of the 10VC compared to the CON and 20VC, while 10VC was not different ($P = 0.25$) from 5VC. No differences ($P \geq 0.16$) in cholesterol content were detected between CON, 5VC, and 20VC.

Western Blot Analysis of μ-Calpain Autolysis and Troponin T Degradation

Calpain-1 autolysis and troponin T degradation data are reported in Figures 1 and 2, respectively. Vitamin C supplementation, regardless of dose, did not affect percentage of the

80-kDa, 78-kDa, or 76-kDa catalytic subunit of the calpain-1 ($P \geq 0.34$) or d 2 or 7 troponin T degradation ($P \geq 0.25$).

Fatty Acid Percentages and Ratios and Individual Fatty Acid Profiles

Fatty acid percentages and calculated ratios and the individual fatty acids (mg of fatty acids/100 g fresh meat) are presented in Tables 4 and 5, respectively. No differences ($P \geq 0.12$) due to treatment were observed among the fatty acid percentages and ratios. Vitamin C supplementation, regardless of dose, increased ($P = 0.02$) the Δ-9-desaturase (C16) index compared to the CON. However, no differences were noted among individual fatty acids C16:0 and C16:1n7 ($P \geq 0.57$) or the lipid content of the steak ($P \geq 0.22$). Vitamin C supplementation did not influence the Δ-9-desaturase (C18) index ($P \geq 0.37$) or the individual fatty acids that contribute to the calculation, C18:0 or C18:1c9 ($P \geq 0.43$). The CLA content of steaks showed a quadratic response ($P = 0.03$) to VC supplementation, which was primarily being driven by lesser ($P \leq 0.04$) CLA concentration of the 10VC treatment compared to the CON, 5VC, and 20VC treatments. No other individual fatty acids were altered ($P \geq 0.13$) due to increasing the VC concentration in the diet.

Correlations

Pearson correlations between color measures (L*, a*, and b*), troponin T degradation (d 2 and 7), μ-calpain autolysis, VE, VC, and cholesterol content, and Ca and Fe concentration were examined. Iron content was negatively associated with troponin T degradation on d 7 (R = -0.48; $P = 0.01$) and a* and b* values (R = -0.63; $P < 0.001$). A positive relationship was identified between d 2 troponin T degradation and the percentage of

the 76-kDa subunit of calpain-1 (R = 0.40; $P = 0.04$). Vitamin E and VC were positively correlated with a* (R= 0.99 and 0.73, respectively; $P < 0.001$) and b* values (R = 0.99 and 0.67, respectively; $P < 0.001$), and VE was positively associated with cholesterol content (R = 0.37; $P = 0.05$) and tended to be positively related to the presence of the 76-kDa subunit of calpain-1 (R = 0.36; $P = 0.06$).

DISCUSSION

Research concerning the incorporation of DDGS to cattle diets has not been specifically focused on the consequences dietary S may be imposing on meat quality, but rather the focus has been in relation to the fat content of DDGS and subsequent impacts on meat quality. However, Pogge & Hansen (2013*a*) have observed that feeding a high S diet (40% DDGS) to feedlot cattle may cause the development of a systemic oxidative environment, based on increased oxidized glutathione in the liver. An oxidative environment can decrease the stability of lipids and color (Greene & Price, 1975) and limit calpain-1 activity, thereby decreasing protein degradation and increasing WBSF (Rowe et al., 2004). However, the incorporation of antioxidants, such as VC or VE, to feedlot diets or during meat processing can increase color and lipid stability (Hood, 1975; Mitsumoto et al., 1991; Harris, Huff-Lonergan, Lonergan, Jones, & Rankins, 2001), as these vitamins are capable of limiting the propagation of reactive oxygen species to prevent damage to cellular components (lipids and proteins) and may aid in prolonging the reducing environment.

Although greater calpain-1 autolysis was associated with greater protein degradation VC supplementation did not affect either trait. These data are dissimilar to the findings previously reported by Pogge et al. (2013), in which consumption of a 0.55% S diet for 149 d

decreased the presence of the 76-kDa subunit of calpain-1 but the addition of 10 g VC·steer^{-1}·d^{-1} to a 0.55% S diet increased the 76-kDa subunit of calpain-1 to values comparable with the low S (0.22% S) treatments. One possible explanation for the lack of similarity between the calpain-1 autolysis data of the two studies may be related to the antioxidant capacity of the cattle on test. High S diets, or the products of S metabolism such as hydrogen sulfide, have been observed to deplete essential antioxidants, such as glutathione and VC (Truong et al., 2006; Pogge & Hansen, 2013*a*), necessary to maintain a reducing environment within cells for protection of enzymes such as calpain-1. Cattle in the present study had greater plasma ascorbate (VC) concentrations, regardless of the dose of supplemental VC (Pogge & Hansen, 2013*b*), compared to the values reported by Pogge & Hansen (2013*a*). While glutathione concentrations were not evaluated in the present study, greater plasma ascorbate concentrations may have contributed to the protection of steers against S-induced oxidative stress and the associated residual effects on calpain-1, as many of the antioxidants in the body are redundant. Additionally, the depletion of glutathione in cultured murine hepatocytes stimulated an increase in the synthesis of ascorbate (Braun et al., 1996). Steers utilized in the Pogge et al. (2013) study received a 0.55% S diet for 37 to 58 d longer than steers in the present study, and this extended duration of S consumption may have contributed to the differences in calpain-1. Consequently, increasing the concentration of VC to steer diets did not impact troponin T degradation or shear force values; however, Fe content of the muscle was negatively associated with troponin T degradation on d 7 ($R = -0.48$). Alternately, while troponin T degradation was not examined by Garmyn et al. (2010), these authors reported no correlation between the Fe content of muscle and shear force values.

Calcium is an essential element for many processes in the body, including bone formation, muscle contraction, and also acts as an enzyme cofactor (Suttle, 2010). Within postmortem muscle, protein degradation is primarily conducted by the Ca-dependent protease calpain-1 (Koohmaraie, 1992). However, no correlation was observed between Ca concentration and the subunits of calpain-1 in the present study. While the influence of dietary S on macro minerals, such as Ca, is less defined, Spears et al. (1985) reported a decreased apparent absorption of Ca in steers consuming S-fertilized grasses. Steers in the present study consuming 5VC and 10VC diets exhibited less Ca than the CON and 20VC treatments, however, as far as the authors are aware no relationship has been identified between Ca and VC. The muscle Ca concentrations of the present study are similar to those reported by Semler (2013), 59.70 mg Ca/kg of tissue; alternately, Ca concentrations reported by Garmyn et al. (2011) and Mello Jr. et al. (2012) were less (37.10 and 24.6 mg/kg, respectively) than those of the current study. Differences in dietary composition or genetic makeup of cattle used in these studies may help explain differences between studies in muscle Ca content. The percent S of the steaks was not different due to treatment, and Haack (2011) and Mello Jr. et al. (2012) reported similar S concentrations of steak samples collected from steers consuming 0 and 35% or 0 to 30% wet distillers grains plus solubles, respectively.

In the present study, the greater quantity of Fe deposited in the muscle may be related to the role of VC in the reduction of ferric to ferrous Fe in the small intestine to facilitate greater Fe absorption (Milne & Omaye, 1980; Van den Berg & Beynen, 1992) and thereby potentially increasing the availability of Fe to be deposited in the muscle. Sorokin, Morgan, & Yeoh (1987) indicated Fe uptake in myotubes occurred through receptor-mediated

endocytosis of the Fe transport protein, transferrin. Richardson, Chikhani, Richardson, & Lane (2012) reported incubation of human skin cells with ascorbate increased Fe uptake from transferrin by 1.5 to 2.5 fold. Pogge et al. (2013) reported an increase in Fe deposition of approximately 2 mg Fe/kg wet tissue in beef LT when 10 g VC·steer^{-1}·d^{-1} was supplemented in a 0.22% S corn-based finishing diet; however, no difference due to VC supplementation was noted in the amount of Fe deposited in the muscle of the 0.55% S treatment. The Fe content reported in the present study are similar to those of Mello Jr. et al. (2012); however, values are approximately 2 to 5 mg Fe/kg tissue greater than values reported by Garmyn et al. (2011) and Pogge et al. (2013).

Meat color has been identified as the most influential component of in the selection process of consumers when purchasing fresh meat products, as consumers associate color of the product with its freshness (Grunert, 1997). The oxidation status of the muscle alters the ligand bound to the heme-Fe in myoglobin and therefore dictates the perceived coloration. Sulfur is able to bind to myoglobin in the place of oxygen to form the green-pigmented sulfmyoglobin (Nichol, Shaw, & Ledward, 1970). Drewnoski et al. (2012) and Pogge & Hansen (2013*a*) reported an increase in the presence of sulfhemoglobin in blood of steers consuming 0.68 and 0.55% S diets, respectively, compared with the low S (0.24 and 0.22% S, respectively) controls; however, in neither of these studies was the presence of sulfmyoglobin evaluated. Because of this relationship between S and Fe it was hypothesized that a* values would be less due to the dilution of the red-pigment by the formation of sulfmyoglobin. While a* values were not different in the present study, Fe content was negatively associated (R = -0.63) with a* and b* values. As muscle Fe content and redness

trend oppositely, this may suggest the pro-oxidant nature of Fe may have deleterious effects on the preservation of steak color.

No other research data are available, as far as the authors are aware, concerning the impact of S-induced oxidative stress on meat color stability; however, it has been established that an oxidative environment, often induced by irradiation, can decrease color stability. The incorporation of VC directly into the meat has been identified as a means to slow redness discoloration and improve color stability for a greater number of days compared to untreated controls (Hood, 1975; Giroux et al., 2001; Nam & Ahn, 2003; Realini, Duckett, & Windham, 2004). It was hypothesized that dietary VC supplementation would increase concentration of VC in the muscle and therefore aid in prolonging color stability. The lack of difference in a* values of the present study may be related to the similar content of VC in the muscle regardless of dietary VC concentration, as no additional VC was applied to the steak surface at any time point during retail display. However, a positive association was identified between a* values and VE (R = 0.99) and VC (R = 0.67), which may suggest the antioxidants present in the muscle are supportive of color maintenance. In the present study, steers supplemented with VC had lesser L* values, indicating darker pigmentation to the meat. Realini et al. (2004) reported the incorporation of VC to ground beef patties from both grass- and grain-fed beef resulted in a similar trend to decrease L* values in ground beef over an 8 d retail display period.

Collagen is one of the most abundant proteins in the body, specifically contributing to the extracellular scaffolding for attachment of cells and anchoring components of the extracellular matrix together. Both Fe and VC are required as enzyme co-factors in the formation of hydroxyproline, a modified amino acid exclusively found in collagen; therefore,

the measure of hydroxyproline can indicate the amount of collagen within a specific tissue. The maturation of collagen fibrils is facilitated by an irreversible transverse cross-link formation, dependent on Cu and VC as co-factors, indicating the quality and stability of the collagen molecule (Kjær, 2004). The hypothesis was that VC inclusion would contribute to greater collagen turnover and decrease shear force values; however, collagen content of steaks in the present study was not affected by VC inclusion.

Distillers grains have traditionally been a source of crude protein and fat; however, in recent years the removal of oil by centrifugation has decreased the percent fat of DDGS by approximately 30% (Lüking & Funsch, 2009). Few research trials have been conducted to examine the impact of low fat DDGS on fatty acid profiles and shelf life; however, Haack (2011) reported a tendency for greater PUFA concentration in steaks from steers fed diets containing 4.7% (low fat wet distillers grains) compared to diets containing 6.9% fat from the inclusion of traditional WDGS. These authors suggest a greater percentage of fat may be escaping hydrogenation in the rumen by its association with the fiber portion of distillers grains, as opposed to the liquid solubles fraction, thereby increasing the availability of PUFA for deposition in the muscle and lipid depots. Pogge et al. (2013) reported VC supplementation to steers consuming DDGS containing a more traditional fat content (9.6% fat of DDGS; 6.0 % fat of the diet) increased the PUFA concentration of steaks. However, in the present study, a low fat (~6-7% fat of DDGS; 5.08% fat of the diet) DDGS source was used throughout the trial, and neither DDGS nor VC altered the fatty acid profiles of steaks. Pogge et al. (2013) reported the supplementation of 10 g $VC \cdot h^{-1} \cdot d^{-1}$ to a 40% DDGS diet increased the index of Δ-9-desaturase (C16) and VC supplementation within the 0.55% S treatment increased the Δ-9-desaturase (C18) index. Similarly, in the present study, the Δ-9-

desaturase (C16) index was greater in the VC supplemented treatments, however, no differences were noted between the C16 and C18 fatty acids that contribute to the index equations.

Lipid oxidation has been directly linked to the PUFA and the heme-Fe content of the muscle (Rhee, Zinprin, Ordonez, & Bohac, 1988). Because PUFA content in the muscle often increases with greater inclusion rates of DDGS to cattle diets, beef products from these cattle may be at a greater risk for lipid oxidation and the subsequent deterioration of meat quality through a decrease in shelf life and development of rancid off-flavors (Greene & Price, 1975; Rhee, Zinprin, Ordonez, & Bohac, 1988; Koger et al., 2010). The addition of ascorbic acid to ground beef has been shown to increase the stability of color pigments, but have limited effects on lipid oxidation (Greene, Hsin, & Zipser, 1971). This limited ability as an antioxidant in lipid is likely due to the water-soluble nature of VC; however, VC is able to donate electrons to oxidized VE (lipid soluble) to its reduced form for continued protection of lipid membranes. Given this relationship it was hypothesized that VC supplementation would increase the availability of VE in the muscle, and as predicted increasing the concentration of supplemental VC increased the concentration of VE in the muscle. This relationship may provide a means to limit the oxidation of muscle pigments and lipids for greater shelf life during muscle storage. The VE concentrations currently reported are similar to the VE concentrations in ground beef from steers consuming differing inclusions of distillers grains (dried and wet; Koger et al., 2010).

Rule, Macneil, & Short (1997) reported a relationship between PUFA and cholesterol content of beef muscle, as cholesterol is incorporated into lipid membranes for stability; however, no such relationship was identified in the present study. The cholesterol content of

steaks in the present study displayed a quadratic response to VC supplementation, as the 5VC and 10VC treatments contained approximately 3 to 5 mg/100 g tissue more cholesterol than the CON and 20VC treatments. Previous research data indicate cholesterol content of beef muscles is not influenced by breed, gender, anatomical location in the body, or dietary modification, and it has been suggested that cholesterol content may be a function of biological need and cellular requirement (Wheeler, Davis, Stoeker, & Harmon, 1987; Bohac & Rhee, 1988).

In conclusion, under the conditions of this study increasing VC supplementation to steers consuming a 0.55% S diet had little to no impact on shear force, markers of protein degradation, redness (a*) and yellowness (b*) values, VC, cholesterol, or collagen content, or fatty acid profiles of the steaks. However, increasing the concentration of supplemented VC linearly increased VE and Fe deposition in the muscle, while decreasing the L* values.

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Table 1. Ingredient composition of finishing diets (% DM basis)

Ingredient	Common Diet[1]
Corn	45.0
Corn dried distiller's grains[2,3]	40.0
Chopped hay	6.5
Corn silage	5.5
Limestone	1.4
Salt	0.3
Vitamin A premix[4]	0.1
Trace mineral premix[5]	0.035
Rumensin90[6]	0.01
Calcium sulfate[7]	0.60
Analyzed composition	
S[8], %	0.54
Calculated composition[9]	
% Lipid	5.08
Vitamin E, IU·kg^{-1} diet DM	304.3

[1]Treatments: CON: control; 5VC: 5 g vitamin C·steer^{-1}·d^{-1}; 10VC: 10 g vitamin C·steer^{-1}·d^{-1}; 20VC: 20 g vitamin C·steer^{-1}·d^{-1}.

[2]Vitashure C (donated by Balchem Corp., New Hampton, NY) replaced distillers grains plus solubles (DDGS), by 0.11 to 0.43% diet DM, to achieve the target level of vitamin C per steer per day.

[3]Four loads of DDGS (POET; Jewell, IA) were used during the trial, S concentrations were: 0.97, 1.04, 0.96, and 0.96% and fat content: 7.83, 6.91, 5.90, and 6.25%.

[4]Vitamin A premix contained 4,400,000 IU·kg^{-1}.

[5]Provided per kg of diet: 30 mg Zn as $ZnSO_4$; 20 mg Mn as $MnSO_4$; 0.5 mg I as $Ca(IO_3)_2(H_2O)$; 0.1 mg Se as Na_2SeO_3; 10 mg Cu as $CuSO_4$; and 0.1 mg Co as $CoCO_3$.

[6]Provided at 27g/909.1 kg diet (donated by Elanco Animal Health).

[7]Calcium sulfate was included at an average of 0.60% diet DM (range of 0.47 to 0.67%), at the expense of DDGS, to achieve targeted S content in the diet.

[8]Sulfur content for the four treatments are averaged across treatment based on repeated measures least squares mean averages throughout the entire study.

[9]Lipid content was calculated from the analyzed lipid content of individual ingredients and vitamin E concentrations were calculated based on NRC values for each ingredient.

Table 2. The effect of supplemental vitamin C (VC), 5 g VC·h^{-1}·d^{-1} (5VC), 10 g VC·h^{-1}·d^{-1} (10VC), and 20 g VC·h^{-1}·d^{-1} (20VC), on shear force and meat color of *longissimus thoracis* collected from steers consuming a 0.55% S diet

	CON	5VC	10VC	20VC	SEM[1]	Contrast Statements[2] *P* values		
						CON vs. VC	Linear VC	Quad VC
Steaks (*n*)	21	21	21	21				
WBSF[3], N	30.79	30.11	31.38	31.47	0.923	0.84	0.43	0.89
Color analysis								
L*[4,5]	32.42	31.10	31.39	31.03	0.342	0.005	0.03	0.14
a*[5,6]	14.93	14.85	14.94	15.24	0.245	0.78	0.30	0.55
b*[5,7]	9.64	9.38	9.46	9.69	0.212	0.59	0.68	0.31
Hue angle	33.16	32.61	32.62	32.42	0.320	0.11	0.16	0.48
Saturation	17.79	17.59	17.71	18.08	0.305	0.99	0.39	0.44

[1]Standard error of the mean.

[2]Contrast Statements: CON vs. VC = no vitamin C vs. vitamin C; Linear VC = linear effect of vitamin C; Quad VC = quadratic effect of vitamin C.

[3]Warner-Bratzler shear force, Newton of force.

[4]Measure of lightness, 0: black and 100: white.

[5]Day: $P < 0.001$; treatment by day: $P \geq 0.58$.

[6]Measure of redness, + values: red, - values: green.

[7]Measure of yellowness, + values: yellow, - values: blue.

Table 3. The effect of supplemental vitamin C (VC), 5 g VC·h^{-1}·d^{-1} (5VC), 10 g VC·h^{-1}·d^{-1} (10VC), and 20 g VC·h^{-1}·d^{-1} (20VC), on nutrient content and mineral concentration of the *longissimus thoracis* collected from steers consuming a 0.55% S diet

	CON	5VC	10VC	20VC	SEM[1]	Contrast Statements[2] *P* values		
						CON vs. VC	Linear VC	Quad VC
Steaks (*n*)	21	21	21	21				
Collagen, mg/g wet tissue	1.09	1.16	0.98	1.22	0.116	0.55	0.36	0.26
Vitamin C, µg/g tissue	22.2	22.0	24.4	23.5	1.75	0.59	0.48	0.64
Vitamin E, µg/g tissue	1.24	1.71	1.45	1.53	0.74	0.001	0.13	0.03
Cholesterol, mg/100 g tissue	54.4	57.4	59.8	54.9	1.45	0.09	0.97	0.008
Mineral[3]								
Fe, mg/kg	16.5	18.6	18.4	19.2	0.87	0.06	0.36	0.39
Ca, mg/kg	57.0	47.1	53.4	56.9	0.03	0.47	0.07	0.07
S, %	0.21	0.2	0.22	0.21	0.05	0.65	0.78	0.28

[1]Standard error of the mean.

[2]Contrast Statements: CON vs. VC = no vitamin C vs. vitamin C; Linear VC = linear effect of vitamin C; Quad VC = quadratic effect of vitamin C.

[3]Mineral concentration of steak is based on wet tissue basis.

Table 4. The effect of supplemental vitamin C (VC), 5 g VC·h^{-1}·d^{-1} (5VC), 10 g VC·h^{-1}·d^{-1} (10VC), and 20 g VC·h^{-1}·d^{-1} (20VC), on fatty acid percentages and ratios of the *longissimus thoracis* collected from steers consuming a 0.55% S diet

	CON	5VC	10VC	20VC	SEM[1]	Contrast Statements[2] *P* values		
						CON vs. VC	Linear VC	Quad VC
Steaks (*n*)	21	21	21	21				
SFA[3], %	43.87	43.64	43.66	43.21	0.347	0.38	0.21	0.88
MUFA[4], %	45.25	45.38	45.55	45.72	0.483	0.60	0.48	0.92
PUFA[5], %	7.01	6.97	6.84	7.03	0.300	0.86	0.96	0.66
PUFA:SFA	0.161	0.161	0.157	0.163	0.0079	0.99	0.81	0.68
n3[6], %	0.470	0.467	0.479	0.457	0.021	0.93	0.70	0.64
n6[7], %	6.09	6.12	6.05	6.19	0.258	0.90	0.79	0.84
n6:n3	13.03	13.15	12.80	13.66	0.350	0.68	0.24	0.31
Δ-9-desaturase (16) index[8]	10.25	10.85	10.79	10.91	0.207	0.02	0.07	0.21
Δ-9-desaturase (18) index[9]	70.32	70.79	71.31	70.98	0.672	0.37	0.50	0.45
AI[10]	0.655	0.663	0.672	0.653	0.0127	0.62	0.87	0.27
Other[11]	3.69	3.82	3.64	3.81	0.186	0.73	0.76	0.87
Lipid, %[12]	6.10	6.11	5.10	6.14	0.516	0.60	0.92	0.22

[1]Standard error of the mean.

[2]Contrast Statements: CON vs. VC = no vitamin C vs. vitamin C; Linear VC = linear effect of vitamin C; Quad VC = quadratic effect of vitamin C.

[3]Saturated fatty acid calculation, sum of: C10:0, C12:0, C13:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C22:0, C23:0, C24:0.

[4]Monounsatruated fatty acid calculation, sum of: C14:1n5, C16:1n7, C17:1n9, C18:1t6 & t9, C18:1t10, C18:1t11, C18:1t12, C18:1t15, C18:1c9, C18:1c11, C18:1c12, C18:1c13, C20:1n11.

[5]Polyunsaturated fatty acid calculation, sum of: C18:2n6, C18:3n3, C18:3n6, C20:2n6, C20:3n6, C20:3n3, C20:4n6, C20:5n3, C22:5n3, C22:6n3, c9-t11 CLA.

[6]Omega 3 fatty acid calculation, sum of: C18:3n3, C20:3n3, C20:5n3, C22:5n3, and C22n3.

[7]Omega 6 fatty acid calculation, sum of: C18:2n6, C18:3n6, C20:2n6, C20:3n6, and C20:4n6.

[8]Δ-9-desaturase (16) index is calculated: C16:1n7 / (C16:0 + C16:1n7) *100.

[9]Δ-9-desaturase (18) index is calculated: C18:1n9 / (C18:0 + C18:1n9) *100.

[10]Atherogenic index is calculated: ((C12:0 + (4 * C14:0) + C16:0) / (% MUFA + % PUFA)).

[11]Other indicates the percent of unidentified peaks.

[12]Percent lipid of steaks based on fatty acid extraction.

Table 5. The effect of supplemental vitamin C (VC), 5 g VC·h^{-1}·d^{-1} (5VC), 10 g VC·h^{-1}·d^{-1} (10VC), and 20 g VC·h^{-1}·d^{-1} (20VC) on individual fatty acids (mg per 100 g fresh meat) of the *longissimus thoracis* collected from steers consuming a 0.55% S diet

	CON	5VC	10VC	20VC	SEM[1]	Contrast Statements[2] *P* values		
						CON vs. VC	Linear VC	Quad VC
Steaks (*n*)	21	21	21	21				
12:0	3.20	3.29	2.87	3.25	0.333	0.87	0.97	0.54
14:0	147.5	158.2	128.3	153.4	14.90	0.96	0.99	0.46
14:1n5	30.5	35.0	27.2	30.4	3.780	0.94	0.69	0.86
16:0	1,512.5	1,522.0	1,261.0	1,498.5	128.12	0.57	0.79	0.25
16:1n7	174.1	191.1	151.8	181.4	15.43	0.97	0.97	0.45
17:0	70.00	68.53	59.24	68.79	6.233	0.54	0.82	0.28
17:1n9	39.39	45.01	33.17	41.30	4.649	0.94	0.92	0.53
18:0	919.7	884.0	740.6	905.5	82.31	0.43	0.84	0.16
18:1[3]	2,544.0	2,560.7	2,121.2	2,563.5	230.3	0.63	0.91	0.25
18:2n6	271.1	278.1	239.5	282.3	18.75	0.84	0.83	0.24
CLA[4]	25.40	24.15	15.94	24.57	2.602	0.22	0.67	0.03
18:3n3	18.72	19.67	15.24	18.06	1.983	0.65	0.61	0.41
18:3n6	1.50	1.21	1.39	1.57	0.220	0.47	0.63	0.13
20:0	5.52	5.33	4.34	5.52	0.562	0.50	0.93	0.16
20:2n5	5.60	6.10	4.94	5.91	0.526	0.94	0.92	0.46
20:3n6	11.88	11.28	11.17	12.00	0.471	0.48	0.70	0.15
20:4n6	32.46	33.20	34.02	33.69	1.337	0.46	0.52	0.58
20:5n3	1.23	1.18	1.52	1.45	0.157	0.41	0.21	0.59
22:5n3	6.55	6.30	6.43	6.61	0.280	0.76	0.73	0.53
24:0	1.41	1.52	1.09	1.35	0.241	0.76	0.70	0.56

[1]Standard error of the mean.

[2]Contrast Statements: CON vs. VC = no vitamin C vs. vitamin C; Linear VC = linear effect of vitamin C; Quad VC = quadratic effect of vitamin C.

[3]C18:1 includes the summation of C18:1c9, C18:1c11, C18:1c12, C18:1c13, C18:1t6, C18:1t9, C18:1t10, C18:1t11, C18:1t12, and C18:1t15.

[4]CLA includes the summation of c9t11CLA and t10c12CLA.

Figure 1.

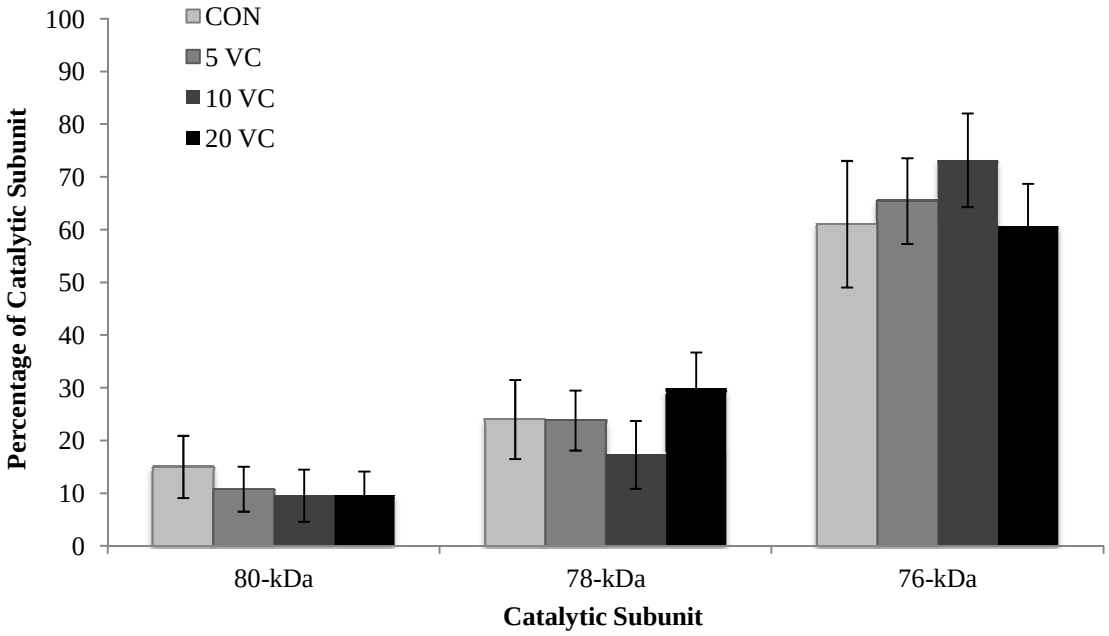

Figure 1. The effect of varying concentrations of supplemental vitamin C (VC), 5 g VC·h^{-1}·d^{-1} (5VC), 10 g VC·h^{-1}·d^{-1} (10VC), and 20 g VC·h^{-1}·d^{-1} (20VC) on calpain-1 autolysis at 2-d postmortem in the *longissimus thoracis* collected from steers (n = 84; 3/pen) consuming a 0.55% S diet. 80 kDa subunit: control vs. vitamin C (P = 0.34); linear effect of vitamin C (P = 0.44); quadratic effect of vitamin C (P = 0.55). 78 kDa subunit: control vs. vitamin C (P = 0.95); linear effect of vitamin C (P = 0.39); quadratic effect of vitamin C (P = 0.15). 76 kDa subunit: control vs. vitamin C (P = 0.51); linear effect of vitamin C (P = 0.95); quadratic effect of vitamin C (P = 0.19). Standard error of the mean: ± 4.47, 6.27, and 6.95 for 80 kDa subunit, 78 kDa subunit, and 76 kDa subunit, respectively.

Figure 2.

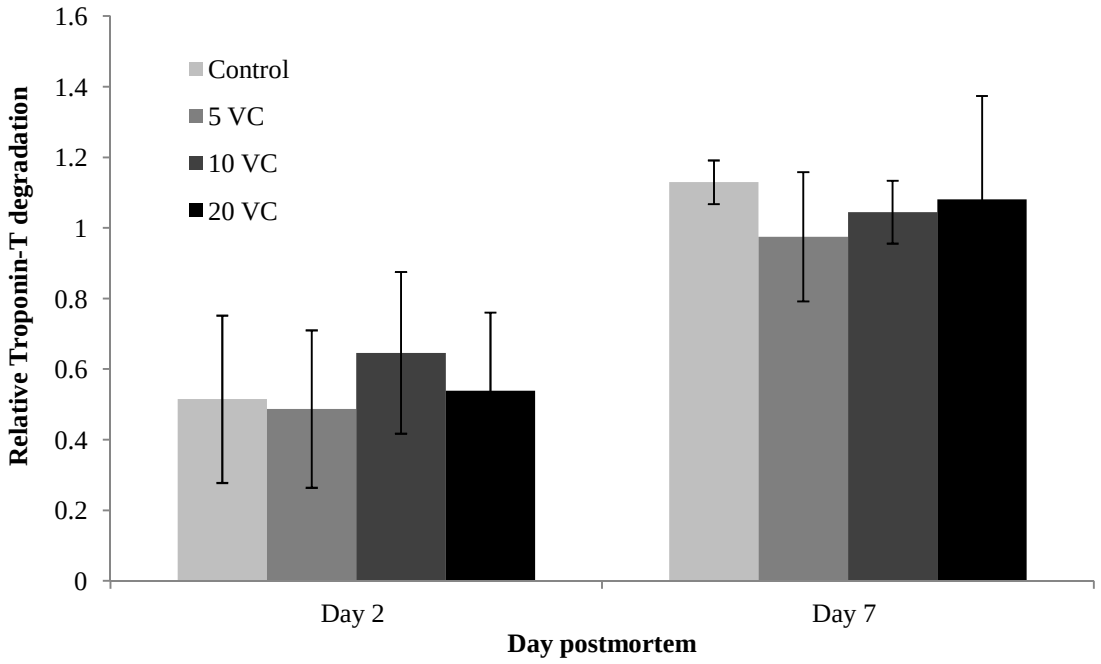

Figure 2. The effect of varying concentrations of supplemental vitamin C (VC), 5 g VC·h^{-1}·d^{-1} (5VC), 10 g VC·h^{-1}·d^{-1} (10VC), and 20 g VC·h^{-1}·d^{-1} (20VC) on troponin T degradation at 2- and 7-d postmortem in the *longissimus thoracis* collected from steers (n = 84; 3/pen) consuming a 0.55% S diet. Day 2 postmortem: control vs. vitamin C (P = 0.56); linear effect of vitamin C (P = 0.57); quadratic effect of vitamin C (P = 0.31). Day 7 postmortem: control vs. vitamin C (P = 0.26); linear effect of vitamin C (P = 0.95); quadratic effect of vitamin C (P = 0.25). Height ratio represents the sample peak height (of the band detected by western blotting) divided by the control sample peak height; greater ratio indicates a greater degradation. Standard error of the mean: ± 0.228 and 0.157 for d 2 and 7 postmortem, respectively.

CHAPTER 8.

IMPACT OF SUPPLEMENTING VITAMIN C FOR 56, 90, OR 127 DAYS ON GROWTH PERFORMANCE AND CARCASS CHARACTERISTICS OF STEERS FED A 0.31 OR 0.59% SULFUR DIET

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ABSTRACT: The objective of this study was to examine differential timing of vitamin C (**VC**) supplementation during the finishing period (for the first 56, 90, or the full 127 d) on growth performance, plasma antioxidants VC and glutathione (**GSH**), and carcass traits of steers receiving a 0.31 or 0.59% S diet. Angus steers (n = 42) were stratified into pens by initial BW (304 ± 13 kg) and Gene-Max marbling score (4.3 ± 0.12), and each pen was randomly assigned to 1 of 7 treatments (2 × 3 plus 1 factorial design; 6 steers/pen, 1 pen/treatment), including: 1) high S (0.59% S) control (**HS CON**), 2) HS CON + 10 g VC·steer^{-1}·d^{-1} for the first 56 d (**HS VC56**), 3) HS CON + 10 g VC·steer^{-1}·d^{-1} for the first 90 d (**HS VC90**), 4) HS CON + 10 g VC·steer^{-1}·d^{-1} for 127 d (**HS VC127**), 5) low S (**LS,** 0.31% S) + 10 g VC·steer^{-1}·d^{-1} for the first 56 d (**LS VC56**), 6) LS + 10 g VC·steer^{-1}·d^{-1} for the first 90 d (**LS VC90**), and 7) LS + 10 g VC·steer^{-1}·d^{-1} for 127 d (**LS VC127**). Jugular blood and ultrasound measures were taken from each steer prior to feeding on d 0, 56, 90, and 127, and liver biopsies and hydrogen sulfide were collected on d 121 and 122. Steers (n = 40) were harvested on d 127, and carcass data were

collected. Individual intake data were collected, thus steer is the experimental unit. Between d 0 and 127 of the study, final live BW, DMI, and G:F were not different ($P \geq 0.19$) as a result of treatment, but ADG ($P = 0.08$) and carcass-adjusted final BW ($P = 0.03$) were greater in the low S steers compared to high S steers. On d 56, plasma ascorbate concentrations of the low S steers tended to be lesser ($P = 0.08$) than the high S steers, but were not different ($P \geq 0.18$) on d 90 or 127. Total GSH was not different ($P \geq 0.40$) due to dietary S concentration on d 56, 90, or 127. Interestingly, the low S steers compared to high S steers tended ($P \leq 0.10$) to have greater oxidized GSH and lesser reduced GSH on d 56, and tended ($P \leq 0.10$) to have lesser oxidized GSH and greater reduced GSH on d 127. Liver Cu, Mn, and Zn were not influenced ($P \geq 0.14$) by treatment; but within the low S diets liver Fe showed a quadratic response ($P = 0.05$) to d of VC supplementation, being greatest in the LS VC90 steers. Ribeye area, marbling score, KPH, and quality grade were not different ($P \geq 0.19$) as a result of diet; but high S steers had less ($P = 0.05$) back fat compared to low S steers. In conclusion, limited effects of differing d of VC supplementation and dietary S were noted among performance and carcass traits of steers.

INTRODUCTION

High S diets can decrease the growth and carcass performance of cattle (Spears et al., 2011; Uwituze et al., 2011; Richter et al., 2012; Pogge and Hansen, 2013*a*). Truong et al. (2006) suggest that S, as hydrogen sulfide (**H_2S**), might be a factor in the development of oxidative stress by depleting body stores of the antioxidant glutathione (**GSH**). A ratio of oxidized-to-reduced GSH exceeding 10% is indicative of oxidative stress (Ithayraja, 2011), and Pogge and Hansen (2013*a*) observed a ratio of 28% oxidized-to-reduced GSH in steers consuming a 0.55% S diet, and suggested these cattle were experiencing some oxidative stress. Given that peak H_2S

production occurs within 10 to 35 d after cattle begin consuming a high S diet (Loneragan et al., 1997; Drewnoski et al., 2012; Loerch et al., 2012; Richter et al., 2012), means to bolster the antioxidant capacity early in the finishing period may lessen or prevent oxidative stress.

Several roles of vitamin C (**VC**) have been identified in the body, including oxidation-reduction reactions and collagen synthesis (Rebouche, 1991). Cattle can synthesize VC in the liver, so no exogenous requirement for VC is specified (NRC, 1996). Cattle requirements for VC are unknown, but plasma VC has been observed to decrease during the finishing period (Takahashi et al., 1999). When supplemented throughout finishing, benefits of supplementing VC may include increased marbling score (Yano, 2001; Pogge and Hansen, 2013a) or ribeye area (**REA**; Oohashi et al., 1999; Pogge and Hansen, 2013b). Because maximal H_2S production occurs early in the finishing period and VC supplementation represents an extra response to the feedlot producer, our objective was to examine differential timing of VC supplementation during the finishing period (for the first 56, 90, or the entire period) on growth performance, plasma antioxidants VC and GSH, and carcass traits of steers receiving a 0.31 or 0.59% S diet.

MATERIALS and METHODS

Procedures and protocols for this experiment were approved by the Iowa State University Institutional Animal Care and Use Committee, protocol number 3-13-7225-B.

Animals and Experimental Design

Forty-two purebred Angus steers (230 to 280 d of age) from Iowa State University's McNay Research Farm (Chariton, Iowa) were transported to the Iowa State University Beef Nutrition Farm (Ames, IA), in April 2013. Steers were started on a common receiving diet for 7

d, followed by a series of three step up diets where corn gradually replaced chopped hay in preparation for the finishing diet (Table 1). Steers were fitted with an electronic identification tag and adapted to the Iowa State University Feed Intake Management System (**FIMS**; 4.69 × 6.86 m; 5.63 m^2/steer). This system allows one animal in a pen to eat at a time, and individual intake data are recorded based on each steer's electronic identification tag.

Prior to the initiation of the study, Gene Max™ (Certified Angus Beef, LLC; Wooster, OH) DNA test for performance and marbling potential of high-percentage Angus cattle were determined of each steer (n = 42). Steers were stratified to treatments (1 pen/treatment, 6 steers per pen) by the average of two consecutive d BW collected on d -5 and -4 (304 ± 13 kg BW) and Gene-Max™ marbling score (4.3 ± 0.12, which included: one 3, two 4, and three 5; scale of 1 to 5, with 5 being the top 20% marbling potential; Certified Angus Beef, LLC; Wooster, OH), and randomly assigned to one of seven treatments arranged in a 2 × 3 plus 1 factorial design (6 steers/pen, 1 pen/treatment). Treatments included: 1) high S (0.59% S) control (**HS CON**), 2) HS CON + 10 g VC·steer^{-1}·d^{-1} for the first 56 d of the finishing period (**HS VC56**), 3) HS CON + 10 g VC·steer^{-1}·d^{-1} for the first 90 d of the finishing period (**HS VC90**), 4) high S diet + 10 g VC·steer^{-1}·d^{-1} for the entire 127 d finishing period (**HS VC127**), 5) low S (**LS**, 0.31% S) + 10 g VC·steer^{-1}·d^{-1} for the first 56 d of the finishing period (**LS VC56**), 6) low S diet + 10 g VC·steer^{-1}·d^{-1} for the first 90 d of the finishing period (**LS VC90**), and 7) low S diet + 10 g VC·steer^{-1}·d^{-1} for the entire 127 d finishing period (**LS VC127**).

Because the receiving and step-up diets contained concentrations of dietary S that ranged from 0.21 to 0.26% S, all steers were limit fed their assigned treatment diet for 5 d prior to the start of the study (d -5 to -1) to decrease the risk of developing S toxicity in steers that were switched to the 0.59% S diet. During the limit feeding period steers were removed from their

respective FIMS pens and placed in a pen (same six steers in a single pen) which allowed all steers access to the bunk at a time, in order to limit the risk of unbalanced diet consumption by steers due to pen social hierarchy. On the first d of limit feeding feed was delivered at 1.75% BW (DM basis) which was increased by 0.25% of BW for each of the four following d. No VC was supplemented during the limit feeding period.

On d -5 steers were de-wormed with Ivomec Eprinex Pour-On for Beef and Dairy (5 mg eprinomectin/mL; Merial Animal Health, Duluth, GA), vaccinated with a modified live virus against bovine viral diarrhea types 1 and 2, infectious bovine rhinotracheitis, parainfluenza-3, and bovine respiratory syncytial virus (Bovi-shield Gold 5; Zoetis, Kalmazoo, MI), and implanted with Component E-S (200 mg Progesterone USP and 20 mg estradiol benzoate; VetLife, Ivy Animal Health, Inc., Overland Park, KS). Steers were subsequently re-implanted 62 d later (on d 56 of the study) with Component TE-IS (80 mg trenbolone acetate and 16 mg estradiol USP).

At the initiation of the study two consecutive d weights were taken (d -1 and 0; BW 311 ± 12.4 kg), steers were moved back to the FIMS pens and a VC premix containing Vitashure C50 (a rumen protected ascorbate, 50% VC product; Balchem Corp., New Hampton, NY) and DDGS was used to introduce VC to the appropriate diets (first d of the study). The inclusion rate of the VC premix was adjusted, based on weekly DMI averages for each treatment when applicable, in order to maintain the designated VC content of 10 g VC in the diet. Two, 25-t semi-truck loads of DDGS (Lincoln Way Energy, Ames, IA), containing 0.32 and 0.33% S and 9.59 and 9.74% fat, respectively, were used in this study. Sodium sulfate was included at 0.28 or 1.64% diet DM in the low S and high S diets, respectively, to maintain a targeted total S

concentration of 0.25 and 0.55% S, respectively. Diets were analyzed to contain 0.31 and 0.59% S for the low S and high S treatments, respectively.

Steers were harvested on d 127 (n = 40 steers; 459 ± 6.0 kg BW) at a commercial packing facility in Denison, IA (Tyson Fresh Meats) when greater than 60% of steers in a pen were estimated by visual appraisal to have at least 1.27 cm of BF. Individual identification was maintained with each carcass following harvest. Carcasses were chilled for 24 h, after which carcasses were ribbed between the 12^{th} and 13^{th} rib and graded according to USDA standards by representatives of the Tri-County Carcass Futurity (Iowa State University Beef Extension, Lewis, IA), who were masked to treatment. Data collected from the harvested animals included HCW, marbling score, BF, KPH, REA, quality grade (**QG**) and yield grade (**YG**). For carcass-adjusted performance data calculation, final BW was determined by dividing the HCW by the average dressing percentage of 63%. To all live BW measures a 4% pencil shrink was applied prior to the calculation of ADG.

Sample Collection

Two consecutive d weights were collected on d -1 and 0, 56 and 57, 90 and 91, and 126 and 127 (final weights). In conjunction with the weigh d, ultrasound measures were conducted on d -5, 56, 90, and 126, capturing REA, percent intramuscular fat of the REA, 12^{th} rib back fat thickness (**BF**), and rump fat thickness image data. Jugular blood was collected from each steer into heparinized tubes (sodium heparin, Becton, Dickinson and Co., Franklin Lakes, NJ) for plasma analysis prior to feeding on d 0, 56, 90, and 126. On d 121 or 122 liver biopsies (n = 24 and 17 steers/d, respectively) and ruminal H_2S measurements (n = 23 and 13 steers/d,

respectively) were collected 6 h post-feed delivery according to the methods described by Engle and Spears (2000) and Drewnoski et al. (2012), respectively.

Individual ingredients and total mixed rations (**TMR**) were sampled weekly for DM determination. Samples were dried in a forced air oven at 70°C for 48 h. Dry matter adjustments were applied to TMR samples by multiplying the percent DM of the appropriate TMR by the as-fed value for each weekly individual intake. Feed efficiency (**G:F**) was calculated from steer weight gain based on the average BW of the two consecutive d weights of d 56 and 57, 90 and 91, and 126 and 127 and total DMI for each interim weight period. Total mixed rations for each treatment were composited by mo, and the monthly composites were then analyzed for S content according to the method described by Richter et al. (2012). Dietary S was calculated by multiplying the percent S by the appropriate TMR samples (DM basis). Supplemental VC intake was calculated by multiplying the percent supplemental VC added in the diet (DM basis) by the weekly DMI.

Analytical Procedures

Jugular blood was transported to the laboratory on ice and was centrifuged at 1,000 × *g* for 10 min at 4°C. Plasma was aliquoted and stored at -80°C prior to the analysis of the plasma ascorbate (Cayman Chemical Company, Ann Arbor, MI; catalog #700420) and GSH (Cayman Chemical Company, Ann Arbor, MI; catalog #703002). Plasma designated for ascorbate analysis was initially prepared and assayed as previously described by Pogge and Hansen (2013a). Plasma for GSH analysis was deproteinated using a 1:1 ratio of metaphosphoric acid and plasma. Samples were vortex and incubated at 25°C for 5 min prior to centrifugation at 2,000 × *g* for 2 min. The supernatant was removed and stored at -20°C until further analysis. Immediately prior

to analysis additional preparation of samples for total GSH and oxidized GSH (**GSSG**) were required. For the determination of total GSH, 10 μL of 4 M triethanolamine was added to a 200 μL aliquot of deproteinated plasma, and a separate aliquot was used for the determination of GSSG, in which 2 μL of 1 M 2-vinylpyridine was added to a 200 μL sample of deproteinated plasma, vortexed, and incubated at 25°C for 60 min. Following these additional preparation steps the assay was then conducted according to the manufacturer's instructions, briefly absorbance of each (total GSH and GSSG) was measured at 405 nm at 5 min intervals over 30 min. Reduced glutathione (**rGSH**) was calculated by subtracting GSSG from total GSH values, and the ratio of oxidized-to-reduced GSH was determined.

Statistical Analysis

Data were analyzed by ANOVA as a completely randomized design using the Mixed Procedure of SAS (SAS Institute Inc., Cary, NC). The model for all analyses included the fixed effect of treatment. The GenMod Procedure of SAS was used to determine differences in the percentages of QG and YG within treatments. Individual steer was the experimental unit for all data analysis (n = 6 per treatment); however, two steers receiving the high S + VC for 56 d were removed from study due to a spinal cord injury (d 104) and death (d 122). Therefore, for this treatment n = 6 steers from d 0 to 90, but n = 4 steers from d 91 to 127. Initial (d 0) plasma ascorbate and GSH concentrations (total, oxidized, reduced, and the ratio of oxidized-to-reduced) were used as covariates for d 56, 90, and 127 ascorbate and GSH analysis (total, oxidized, reduced, and the ratio of oxidized-to-reduced). Six single df contrast statements were constructed prior to analysis: 1) high S diets vs. low S diets; 2) HS CON vs. VC supplemented treatments within the high S treatment; 3) linear effect of days of VC supplementation within high S diets;

4) quadratic effect of days of VC supplementation within the high S diets; 5) linear effect of days of VC supplementation within the low S diets; 6) quadratic effect of days of VC supplementation in LS diets. Procedure CORR of SAS was used to generate Pearson correlation coefficients to determine the relationship between plasma ascorbate, G:F, marbling score, BF, GSH concentrations (total, oxidized, and reduced). Significance was declared at $P \leq 0.05$ and tendencies were declared from $P = 0.06$ to 0.10. The values reported in the tables are least squares means.

RESULTS

Intake and Performance

Steer intake, performance, and S and VC intakes are reported in Table 2. By design initial BW were not different ($P = 0.24$) due to treatment, and BW did not differ on d 56 ($P = 0.17$) or d 127 ($P = 0.19$). However, d 90 BW tended to be greater ($P = 0.09$) in the low S steers compared to the high S steers. Dry matter intake was not different due to S content or VC inclusion to diets between d 0 and 56 ($P \geq 0.18$), d 0 and 90 ($P \geq 0.25$), or d 0 and 127 ($P \geq 0.21$). Steers consuming the low S diets had greater ($P < 0.01$) daily gains between d 0 and 56 and d 0 and 90, and tended to be greater ($P \leq 0.09$) between d 56 and 90 and 0 and 127 compared to the high S steers. Feed efficiency was not different between d 0 and 56 ($P = 0.13$) or d 0 and 127 ($P = 0.51$), but between d 0 and 90 steers consuming the low S diets tended ($P = 0.10$) to be more efficient than the high S steers. Dry matter intake by the high S steers showed a quadratic response ($P = 0.05$) to d of VC supplementation between d 90 and 127, which is driven by the lesser intake of the HS VC56 ($P = 0.08$) and HS VC90 ($P = 0.03$) treatments compared to HS VC127 during this time.

Average daily supplemental VC intakes were not different ($P \geq 0.80$) between VC-supplemented treatments, during any period of the trial. By design, the low S steers consumed fewer ($P < 0.01$) g S/d than the high S steers at all time points. As a marker of ruminal S metabolism, ruminal H_2S concentrations were measured on d 121 or 122 of the trial and were found to be lesser ($P < 0.001$) in the low S steers (0.071 g/m^3) compared to the high S steers (0.314 g/m^3). Days of VC supplementation within the low S diet did not affect ($P \geq 0.47$) S intake. While DMI was not different ($P \geq 0.46$) in steers consuming high S diets (control and VC supplemented) between d 0 and 90 or 0 and 127, increasing the d of VC supplementation tended to increase ($P \leq 0.10$) S intake after 56 d on feed.

Carcass-adjusted final BW were greater in the low S ($P = 0.03$) steers compared to the high S, but ADG and G:F were not different ($P \geq 0.13$) were not different due to treatment.

Plasma Ascorbate and Glutathione

Plasma ascorbate (μg/L) and glutathione (μM/L) concentrations are reported in Table 3. Plasma ascorbate concentration was not different ($P \geq 0.23$) due to dietary S concentration on d 0, 90, or 127. On d 56, the low S steers tended ($P = 0.06$) to have a lesser plasma ascorbate concentration compared to the high S steers, but were not different ($P \geq 0.23$) from the high S steers throughout the remainder of the study. However, on d 127 within the low S diet, increasing d of VC supplementation tended to linearly increase ($P = 0.09$) plasma ascorbate concentrations. Interestingly, plasma ascorbate on d 127 was positively correlated (R = 0.43; $P < 0.005$) to feed efficiency between d 90 and 127, while no correlation was noted between plasma ascorbate and feed efficiency on d 0 and 56 (R = -0.09; $P = 0.57$) or d 56 to 90 (R = 0.07; $P = 0.60$).

Initial plasma tGSH and rGSH concentrations were greater ($P \leq 0.02$) in the low S treatments compared to the high S treatments, and total GSH and rGSH displayed a quadratic effect ($P \leq 0.06$) within the low S diet, which is being driven by a tendency for greater ($P \leq 0.10$) total GSH of LS VC56 steers. On d 56, tGSH was greater ($P = 0.01$) in the VC supplemented steers within the high S treatment compared to the HS CON. A tendency ($P = 0.08$) for greater GSSG, a tendency for lesser ($P = 0.10$) rGSH, and a tendency for a greater ($P = 0.08$) GSSG:GSH was noted in the low S steers compared to high S steers on d 56. Conversely, on d 90 the low S steers had lesser ($P = 0.01$) GSSG content compared to high S steers, while no differences ($P \geq 0.18$) were noted in tGSH, rGSH, or GSSG:GSH. While no differences ($P \geq 0.10$) in tGSH were noted between low S and high S steers on d 127, GSSG tended to be lesser ($P = 0.10$) and rGSH was greater ($P = 0.05$) in the low S steers compared to high S steers.

Liver Mineral Concentrations

Liver mineral concentrations are reported in Table 4. Liver copper, Mn, and Zn concentrations on d 122 were not affected ($P \geq 0.14$) by dietary S concentration or days of receiving supplemental VC. Liver Fe concentration within the high S treatments were not altered ($P \geq 0.14$) due to VC supplementation; however, a quadratic effect ($P = 0.05$) of liver Fe concentration within the low S treatments, which is primarily driven by the ~47 mg/kg increase in the Fe concentration of the LS VC90 treatment compared to the LS VC56 and LS VC127 treatments.

Ultrasound Measures

Ultrasound measures of percent intramuscular fat of the REA, REA, BF, and rump fat are reported in Table 5. Intramuscular fat on d 56 tended to decrease ($P = 0.07$) as the d of VC supplementation within the high S treatment increased, and a tendency for a quadratic effect ($P \leq 0.07$) of d of VC supplementation within the low S treatment was noted on d 90 and 127, which is likely being driven greater intramuscular fat in the LS VC90 treatment compared to LS VC56 and LS VC127 treatments. Additionally, intramuscular fat within the high S treatments tended ($P = 0.07$) to be greater in the HS CON compared to the VC supplemented steers on d 127. Ribeye area was not different ($P \geq 0.14$) on d 0, 56, or 90 between the low S and high S treatments. A tendency for a quadratic effect ($P = 0.09$) of REA on d 56 was noted within the low S treatments, which may be due to the lesser REA of the LS VC90 steers compared to the LS VC56 steers. On d 90, REA linearly increased ($P = 0.05$) as d of VC supplementation increased within the high S treatments. On d 127, the low S steers had a greater REA ($P = 0.003$) compared to the high S steers, and within each concentration of S a tendency ($P \leq 0.08$) for a quadratic effect of d of VC supplementation was noted. These tendencies within the respective concentration of S were driven by the lesser ($P = 0.04$) REA of the LS VC90 compared to the LS VC56, while HS VC56 displayed a lesser REA ($P \leq 0.09$) compared to the other three high S treatments. Twelfth rib BF was not different on d 0 or 127, but on d 56 ($P = 0.10$) and 90 ($P = 0.004$) was greater in the low S steers compared to high S steers. Rump fat thickness was not altered due to dietary S concentration or d of VC supplementation ($P \geq 0.12$) during any period of the trial.

Carcass Characteristics

Carcass characteristics are reported in Table 6. Dietary S content and VC supplementation did not alter ($P \geq 0.12$) HCW. Dressing percentage was lesser ($P = 0.05$) in the high S treatments supplemented with VC compared to the HS CON, and increasing the days of VC supplementation tended to linearly ($P = 0.06$) decrease dressing percentage. No difference ($P \geq 0.14$) in dressing percentage was noted within the low S treatment. The high S steers displayed a lesser ($P = 0.05$) BF thickness compared to the low S steers. Within the high S treatment a tendency for a quadratic effect of days of VC supplementation was observed, which is primarily being driven by the lesser BF of the 56 and 90 d VC treatments. No difference in BF was noted due to VC supplementation in the low S ($P \geq 0.19$) treatments. Ribeye area ($P \geq 0.19$), marbling score ($P \geq 0.33$), KPH ($P \geq 0.25$), and QG ($P \geq 0.37$) were not different due to S content or d of VC supplementation.

Yield grade tended ($P = 0.10$) to be lesser in the high S treatment compared to the low S steers, and increasing the number of days VC was supplemented within the high S treatment tended ($P = 0.09$) to increase YG. Yield grade 2 carcasses from steers fed the high S diet showed a tendency ($P = 0.06$) for a quadratic response to d of VC supplementation, which is driven by the greater percent of YG-2 carcasses from the HS VC56 and HS VC90 steers compared to the HS VC127 steers. The LS VC127 steers tended ($P = 0.07$) to have a lesser percentage of YG 4 and 5 carcasses compared to the LS VC56 and LS VC90 steers. The HS CON steers tended ($P = 0.10$) to have a greater percentage of YG-1 and Select carcasses compared to the high S steers supplemented with VC. Increasing the d of VC supplementation within the high S diet tended ($P = 0.09$) to linearly decrease the percentage of steers grading high Choice, and the percentage of carcasses grading average Choice was greater ($P = 0.03$) in the low S steers compared to high S,

and a quadratic tendency ($P = 0.09$) was noted within the high S diet, which is driven by the HS VC56 treatment having 0% average Choice. The VC supplemented steers within the high S diet tended to have a greater ($P = 0.10$) percentage of steers grading low Choice compared to the HS CON. No differences ($P \geq 0.20$) in the percentage of cattle grading low Choice, average Choice, and high Choice were observed with the low S treatments.

DISCUSSION

The use of supplemental nutrients in feedlot cattle diets introduces new expenses for producers, which may discourage their use; however, identifying the optimal timeframe to incur maximal benefits from the supplement could lessen the cost experienced by producers. We have previously observed some benefits of supplementing VC to cattle consuming high S diets (Pogge and Hansen, 2013a,b); however, it is understood that the greatest stress from high S diets may be occurring early in the finishing period as ruminal metabolism of S results in peak H_2S production (Drewnoski et al., 2012). Therefore, this work was undertaken to determine if VC supplementation for shorter periods of time would still have benefits, while potentially saving money for the beef producer.

Dietary S concentrations exceeding 0.46% have been documented to hinder steer performance, specifically decreasing DMI and subsequently ADG and final BW (Spears et al., 2011; Uwituze et al., 2011; Richter et al., 2012; Pogge and Hansen, 2013*a*). In the present study, steers consuming a high S diet displayed a lesser ADG, while DMI and G:F were not different due to treatment. Our results are similar to those reported by Richter et al. (2012), who showed that yearling steers consuming a 0.6% S diet had an 11% (0.17 kg/d) decrease in ADG compared to steers consuming a diet containing 0.3% S. Additionally, increasing the number of d that

steers consume a high S diet has been shown to decrease liver Cu concentrations of cattle (Underwood and Suttle, 1991; Spears et al., 2011; Pogge and Hansen, 2013a), and this decrease in liver Cu may be further exacerbated by the addition of VC to the diet (Van den Berg et al., 1994; Milne and Omaye, 1980; Pogge and Hansen, 2013a). Surprisingly, in the present study neither dietary S nor supplemental VC had marked effects on liver Cu concentrations.

Vitamin C supplementation did not alter performance in the present study between d 0 and 90, but DMI within the high S steers showed a quadratic response to d of VC supplementation between d 90 and 127. Interestingly, the HS VC56 and HS VC90 steers consumed approximately 0.5 to 1.2 kg/d less than the HS CON and HS VC127 treatments, respectively. This decreased DMI tended to translate to a lesser ADG during d 90 to 127 by the steers that were not receiving VC during this period compared to high S steers receiving VC for the entire finishing period. Also during this period of time, regardless of dietary S concentration in the diet, ADG was approximately 50% of the values observed in the previous period (d 56 to 90), which may be attributed to a lesser DMI between d 99 to 104 due in part to a period of extreme heat after a relatively cool summer (six consecutive d with temperatures greater than 32°C).

The limited effect of VC supplementation on steer performance is consistent with the results of Yano (2002), supplementing 40 mg VC·kg $BW^{-1}·d^{-1}$ (as a rumen protected source) to Japanese black steers (12 to 24 mo of age) for 365 d, and Pogge and Hansen (2013a), supplementing 10 g VC·$steer^{-1}·d^{-1}$ (same VC source as in the present study) for 149 d to calf-fed steers (approximately 8 mo of age). Alternately, Pogge and Hansen (2013b) indicated increasing the dose of supplemental VC (0, 5, 10, or 20 g VC·$steer^{-1}·d^{-1}$; same source as the present study) to yearling steers consuming a 0.55% S diet linearly decreased DMI while tending to increase

feed efficiency. While no treatment differences were noted in feed efficiency in the present study, plasma ascorbate concentrations on d 127 were positively correlated with feed efficiency between d 90 and 127, though not during other periods of the current study.

Bottje and Carstens (2009) suggested that differences in overall animal feed efficiency might be related to mitochondrial efficiencies, as electrons leaking from the electron transport chain (**ETC**) can increase the risk of reactive oxygen species (**ROS**) formation, which can damage protein, lipid, and DNA, by oxidation. Because ascorbate and GSH are major intracellular (and mitochondrial) antioxidants and both are involved in the regeneration of the other to its reduced form, it may be considered that a means to increase the antioxidant capacity of cells (and mitochondria) would impede the formation of ROS and lessen the damage to intracellular components, which may aid in improving efficiency of the animal.

Increasing the sulfate concentration in the diet can bombard cattle with excess S in the rumen, as sulfate is reduced to sulfide and sulfide may be absorbed across the rumen wall or inhaled as eructated H_2S. As we and others have indicated, the peak in H_2S production, resulting from ruminal metabolism of sulfate, occurs within the first 10 to 35 d of cattle consuming a high concentrate, high S diet (Loneragan et al., 1997; Drewnoski et al., 2012; Loerch et al., 2012; Richter et al., 2012; Pogge and Hansen, 2013a). Because excess S can be cleared by conjugation with GSH, during this initial period of excess S exposure a decrease in GSH may occur. Truong et al. (2006) suggested that the depletion of GSH, as a result H_2S exposure, might be contributing to the development of oxidative stress. Briefly, H_2S can impede the activity of cytochrome *c* oxidase of the ETC and increase the release of Fe from the storage protein ferritin, thus increasing the production of ROS. A greater accumulation of ROS can overwhelm the antioxidant capacity of the cell, thus contributing to oxidative stress (Truong et al., 2006).

In the present study, the high S steers tended to have less rGSH and greater GSSG concentrations compared to the low S steers on d 56 and 127. These data may help support the hypothesis that increased exposure to H_2S depletes GSH and that a lesser availability of GSH for other antioxidant functions may contribute to the development of oxidative stress (Truong et al., 2006). As aforementioned, VC and GSH share a regenerative relationship, and it was hypothesized that supplementing VC to steers consuming a high S diet may contribute to the overall antioxidant capacity and provide a sparing mechanism for GSH. In support of this hypothesis, on d 56, the high S steers that were supplemented with VC had greater total and reduced GSH concentrations compared to the HS CON. Also, while no correlation was observed between GSH and plasma ascorbate, on d 56, the tendency for greater circulating ascorbate in the high S steers may have contributed to the tendency for a lesser ratio of oxidized-to-reduced GSH compared to the low S steers. However, beyond d 56, this trend is no longer recognized.

Circulating ascorbate is extremely variable within cattle, as serum ascorbate concentrations measured in Japanese black cattle at harvest ranged from 810 to 16,784 µg/L (Takahashi et al., 1999). These authors subsequently identified a general trend for plasma ascorbate to decrease during the finishing period, regardless of initial ascorbate concentrations. Pogge and Hansen (2013a) reported the addition of high concentrations of S to finishing steer diets magnified this decreasing trend in plasma ascorbate of finishing cattle fed a high S diet (45% decrease over the first 90 d), specifically within the first 90 d. In the present study, plasma ascorbate concentrations decreased approximately 30%, regardless of dietary S concentration. Tsao and Young (1990) indicated that the supply of exogenous VC (dietary) decreased the endogenous production of VC in the liver of mice, a species capable of synthesizing VC. While similar research has not been conducted in cattle it is reasonable to assume a similar interaction

may be occurring. With a greater number of d consuming VC, a linear increase in plasma ascorbate was noted on d 127 in the low S steers, which may support a potential dependency on exogenous VC in the low S steers. Alternately, the lack of differences due to d of VC supplementation within steers consuming a high S diet may indicate that any extra VC in circulation was being utilized to combat oxidative stress.

In the present study, plasma ascorbate values are similar to those reported by Hidiroglou et al. (1977) and Pogge and Hansen (2013b); however, in these studies no decrease in plasma ascorbate was observed. The lack of a decrease in ascorbate concentrations may be related to the age of the cattle used in the respective studies, yearlings by Hidiroglou et al. (1977) and Pogge and Hansen (2013b) compared to calf-fed steers (8 mo of age) utilized in the present study and by Pogge and Hansen (2013a). The differing response in plasma ascorbate during the finishing period may indicate that calf-fed cattle have a greater requirement of VC compared to yearling steers. However, no data are available to support or contradict this idea. Within the Japanese black breed of cattle Matsui (2012) reported that plasma ascorbate concentrations of calves, fattening heifers and steers, and replacement heifers remained moderately stable up to approximately 13 mo of age, after which circulating VC progressively decreased. Matusi (2012) proposed that plasma ascorbate is decreasing due to the fattening of cattle rather than age, which may be reflective of the greater number of days on feed and generally greater marbling potential of the Japanese black breed of cattle in comparison to typical United States feedlot cattle. In the present study, the relationship between plasma ascorbate and GSH (d 127), marbling score, and BF were examined, as greater quantity of lipid may increase the risk for oxidation and contribute to the depletion of antioxidants; however, no relationship was observed among any of the measures.

While the exact allocation of VC to biological processes during the finishing period have not been characterized, the downward trend in circulating VC during the first 90 d, specifically in calf-fed steers, may be related to support of overall growth and development of the skeleton, adipose tissue, and muscle. The contribution of VC to these components of growth may be associated with collagen synthesis and its incorporation into the extracellular matrix, as VC is an essential cofactor in the hydroxylation reactions for collagen production (Aberle et al., 2001). The extracellular matrix functions to providing scaffolding for cellular attachment during development and to facilitate the interaction of cells with their environment. In contrast to yearling cattle that enter the feedyard with a nearly mature frame size, calf-fed cattle require more skeletal growth during the finishing period to reach mature size. This greater growth requirement may correspond to a greater need for type II fibril collagen, which forms the initial framework of the bone prior to mineralization (Aszódi et al., 2001). Additionally, the connective tissue surrounding muscle fibers and adipose tissue must be altered to facilitate hypertrophy. Nakajima et al. (1998) reported greater gene expression of type IV network-forming collagen during adipocyte differentiation. The rapid growth observed by feedlot cattle, especially when cattle receive implants (Lawrence et al., 2001), may be increasing the collagen turnover and thus the requirement for VC. Steers in the present study were re-implanted on d 56, which may have contributed to the decrease in plasma ascorbate during the initial 90 d period.

The benefits of VC supplementation to feedlot diets have been primarily noted in carcass characteristics and meat quality; however, the timing of VC supplementation to cattle diets may influence these benefits. Oohashi et al. (1999) reported no difference in BF thickness of Japanese black steers consuming 50 g VC·steer^{-1}·d^{-1} for differing time periods throughout a 15 mo finishing period (the initial 7 mo, latter 8 mo, or the entire 15 mo), but VC inclusion to the diet

during the first 7 mo of finishing increased lipid accumulation in steers while VC supplementation during the latter 8 mo period increased ribeye area, which authors attribute to a potential growth promoting effect of VC. Similarly, the supplementation of VC for the entire finishing period of calf-fed steers (149 d; Pogge and Hansen, 2013a) and yearling steers (102 d; Pogge and Hansen, 2013b) consuming a high S (0.55%) diet increased marbling score and BF thickness in calf-fed steers (Pogge and Hansen, 2013a) and REA in yearling steers (Pogge and Hansen, 2013b). Dissimilarly, no effects of supplemental VC on marbling score or REA were noted in the present study. Back fat thickness in the present study was not different due to VC supplementation, but rather BF was altered by the concentration of S in the diet. A trend for greater BF thickness by the low S steers was noted on d 56 of the study by ultrasonography and the trend continued throughout the remainder of the study.

The lack of VC or dietary S effects on marbling score and REA in the current study may be attributed to the source of cattle selected for the study and concentrations of plasma ascorbate. The steers used in the present study were from an Angus cowherd that has undergone many years of intensive genetic selection for greater quality grades (marbling). Therefore, the similar marbling scores of cattle consuming the low and high S diets may not accurately show the impact that dietary S or supplemental VC could have on marbling scores. Interestingly, while all steers had a greater genetic predisposition for marbling, the HS CON was the only treatment in which any steers graded Select (17% of the HS CON). Additionally, the plasma ascorbate concentrations reported by Pogge and Hansen (2013a) were approximately 2-fold less (averaged 550 µg/L over 149 d) than those in the present study (averaged 1,450 µg/L over 127 d), but marbling score was increased a quality grade when VC was supplemented in the high S diet (0.55% S; Pogge and Hansen, 2013a). In contrast, plasma ascorbate concentrations greater than

1,100 µg/L in yearling steers (Pogge and Hansen, 2013b) and calf-fed steers in the present study consuming a high S diet resulted in no effect on marbling score, which may indicate there is a threshold for plasma ascorbate concentration to influence marbling potential. Further work is needed to clarify the relationship between circulating ascorbate and marbling development in beef animals.

In conclusion, under the conditions of this study limited effects of dietary S and supplemental VC were noted on performance and carcass traits, which may be related to the source of cattle used in the study or a threshold concentration of circulating VC that might influence marbling potential. Additionally, the regenerative relationship between VC and GSH may indicate VC supplementation could provide a sparing mechanism for GSH if cattle experience oxidative stress when consuming high S diets.

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Table 1. Ingredient composition of finishing diets (% DM basis)

Ingredient	Low S Diet[1,2]	High S Diet[1,2]
Corn	42.92	42.92
Corn dried distiller's grains[3]	39.73	38.36
Corn silage	15.0	15.0
Limestone	1.62	1.62
Salt	0.31	0.31
Vitamin A premix[4]	0.10	0.10
Trace mineral premix[5]	0.035	0.035
Rumensin90[6]	0.014	0.014
Sodium sulfate[7]	0.275	1.64
Analyzed composition		
S[8], %	0.31	0.59
Calculated composition		
Lipid[9], %	6.23	6.18
Vitamin E[10], IU·kg^{-1} diet DM	343.0	343.5

[1]Treatments: Low S Diet: low S (0.25% S) + 10 g vitamin C·steer^{-1}·d^{-1} control and High S Diet: high S (0.55% S) control, 0 VC and a high S (0.55% S) + 10 g vitamin C·steer^{-1}·d^{-1}.

[2]Vitashure C (donated by Balchem Corp., New Hampton, NY) replaced distillers grains plus solubles (DDGS), by 0.16 to 0.21% diet DM, to achieve the 10 g vitamin C·steer^{-1}·d^{-1}.

[3]Two loads of DDGS (Lincoln Way Energy; Ames, IA) were used during the trial, S concentrations were: 0.33 and 0.32% and fat content: 9.59 and 9.74%.

[4]Vitamin A premix contained 4,400,000 IU·kg^{-1}.

[5]Provided per kg of diet: 30 mg Zn as $ZnSO_4$; 20 mg Mn as $MnSO_4$; 0.5 mg I as $Ca(IO_3)_2(H_2O)$; 0.1 mg Se as Na_2SeO_3; 10 mg Cu as $CuSO_4$; and 0.1 mg Co as $CoCO_3$.

[6]Provided at 27g/907.18 kg diet (donated by Elanco Animal Health, Greenfield, IN).

[7]Sodium sulfate was included at 0.275 or 1.64% diet DM, at the expense of DDGS, to achieve targeted S content (0.25 or 0.55% S, respectively) in the diet.

[8]S content for treatments are averaged across treatment based on least squares mean averages throughout the entire study.

[9]Lipid content was calculated from the analyzed lipid content of DDGS and corn silage, and the NRC value of corn was used.

[10]Vitamin E concentrations were calculated based on NRC values for each ingredient.

Table 2. The influence of supplementing of 10 g vitamin C (VC)·steer^{-1}·d^{-1} for 0, 56, 90, or 127 of the finishing period on body weight, performance, sulfur and VC intakes, and carcass based performance of steers consuming a 0.31 or 0.59% sulfur diet

	HS Con[1]	HS VC56[1]	HS VC90[1]	HS VC127[1]	LS VC56[1]	LS VC90[1]	LS VC127[1]	SEM	1[2]	2[2]	3[2]	4[2]	5[2]	6[2]
BW[3], kg														
d 0	308	311	311	310	312	314	310	12.4	0.24	0.87	0.61	0.69	0.99	0.97
d 56	394	396	396	399	412	410	408	12.9	0.17	0.81	0.79	0.99	0.84	0.96
d 90	469	466	474	474	490	487	489	13.5	0.09	0.87	0.73	0.86	0.97	0.88
d 127	507	497	503	525	525	519	531	19.7	0.19	0.95	0.49	0.34	0.79	0.64
DMI, kg/d														
d 0 to 56	9.90	9.42	10.58	9.74	10.30	10.37	10.39	0.425	0.18	0.98	0.78	0.88	0.88	0.96
d 56 to 90	11.66	10.90	12.60	11.77	11.59	11.99	12.13	0.404	0.58	0.84	0.35	0.73	0.35	0.78
d 90 to 127	9.37	8.93	8.80	10.07	9.25	9.64	9.97	0.488	0.30	0.83	0.36	0.05	0.21	0.92
d 0 to 90	10.44	9.85	11.20	10.37	10.66	10.85	10.91	0.385	0.25	0.93	0.58	0.98	0.64	0.89
d 0 to 127	10.13	9.51	10.50	10.29	10.25	10.50	10.64	0.434	0.21	0.94	0.46	0.43	0.44	0.88
ADG, kg/d														
d 0 to 56	1.70	1.77	1.80	1.80	2.00	1.94	1.94	0.071	<0.01	0.16	0.18	0.62	0.43	0.66
d 56 to 90	2.29	2.14	2.34	2.29	2.36	2.34	2.45	0.095	0.09	0.72	0.77	0.44	0.49	0.61
d 90 to 127	1.03	1.02	0.79	1.37	0.94	0.85	1.12	0.192	0.52	0.87	0.31	0.10	0.40	0.36
d 0 to 90	1.90	1.88	1.98	1.95	2.11	2.07	2.10	0.055	<0.01	0.49	0.29	0.89	0.93	0.50
d 0 to 127	1.65	1.63	1.64	1.79	1.77	1.72	1.82	0.081	0.08	0.65	0.19	0.21	0.58	0.35
G:F														
d 0 to 56	0.172	0.189	0.174	0.186	0.195	0.186	0.190	0.0091	0.13	0.31	0.45	0.74	0.71	0.66
d 56 to 90	0.197	0.195	0.187	0.195	0.203	0.197	0.203	0.0084	0.24	0.65	0.71	0.64	0.99	0.53
d 90 to 127	0.108	0.111	0.088	0.136	0.101	0.086	0.112	0.0172	0.30	0.81	0.38	0.18	0.57	0.23
d 0 to 90	0.182	0.192	0.179	0.189	0.199	0.191	0.195	0.0074	0.10	0.59	0.74	0.92	0.78	0.52
d 0 to 127	0.162	0.172	0.157	0.174	0.173	0.163	0.173	0.0083	0.51	0.52	0.48	0.70	0.99	0.24

	HS Con[1]	HS VC56[1]	HS VC90[1]	HS VC127[1]	LS VC56[1]	LS VC90[1]	LS VC127[1]	SEM	1[2]	2[2]	3[2]	4[2]	5[2]	6[2]
Intakes														
S, g·steer^{-1}·d^{-1}														
d 0 to 56	56.07	57.10	61.18	58.86	32.48	33.11	32.73	2.018	<0.01	0.21	0.17	0.59	0.94	0.84
d 56 to 90	58.78	54.96	64.48	60.97	29.16	30.63	30.09	1.759	<0.01	0.51	0.08	0.50	0.47	0.78
d 90 to 127	57.53	55.06	54.22	64.08	26.28	27.47	27.71	2.586	<0.001	0.92	0.10	0.01	0.64	0.85
d 0 to 90	57.11	56.28	62.45	59.68	31.20	32.16	32.07	1.771	<0.001	0.26	0.10	0.90	0.78	0.80
d 0 to 127	57.25	56.21	60.16	60.90	29.84	30.85	30.85	1.633	<0.01	0.33	0.06	0.39	0.67	0.79
VC[4], g·steer^{-1}·d^{-1}														
d 0 to 56	--	10.07	10.62	10.32	10.72	10.76	10.82	0.405	--	--	--	--	--	--
d 56 to 90	--	--	10.63	10.71	--	10.41	10.76	0.325	--	--	--	--	--	--
d 90 to 127	--	--	--	9.81	--	--	9.32	0.258	--	--	--	--	--	--
d 0 to 90	--	10.07	10.62	10.47	10.72	10.63	10.80	0.356	--	--	--	--	--	--
d 0 to 127	--	10.07	10.62	10.47	10.72	10.63	10.79	0.357	--	--	--	--	--	--
Carcass-adjusted performance[5]														
Final BW, kg	513	501	503	517	532	512	530	11.2	0.03	0.57	0.84	0.17	0.92	0.09
ADG, kg/d	1.65	1.64	1.65	1.79	1.77	1.72	1.82	0.091	0.13	0.64	0.25	0.31	0.63	0.40
G:F	0.162	0.172	0.158	0.174	0.173	0.163	0.173	0.0091	0.59	0.51	0.51	0.81	0.98	0.29

[1]Treatments: HS Con: high S control; HS VC56: high S + 10 g VC·steer^{-1}·d^{-1} for the first 56 d of study; HS VC90: high S + 10 g VC·steer^{-1}·d^{-1} for 90 the first 90 d of the study; HS VC127: high S + 10 g VC·steer^{-1}·d^{-1} for the entire finishing period (127 d); LS VC56: low S + 10 g VC·steer^{-1}·d^{-1} for the first 56 d of study; LS VC90: low S + 10 g VC·steer^{-1}·d^{-1} for 90 the first 90 d of the study; LS VC127: low S + 10 g VC·steer^{-1}·d^{-1}for the entire finishing period (127 d).

[2]Contrast statements: 1: high S diets vs. low S diets; 2: control (0 VC) vs. VC supplemented treatments within the high S treatment; 3: linear effect of days of VC supplementation within high S diets; 4: quadratic effect of days of VC supplementation within the high S diets; 5: linear effect of days of VC supplementation within the low S diets; 6: quadratic effect of days of VC supplementation in LS diets.

[3]A 4% pencil shrunk was applied to body weight (BW).

[4]Vitamin C daily intakes, d 0 to 56 treatment $P = 0.81$; d 0 to 90 treatment $P = 0.80$; d 0 to 127 treatment $P = 0.80$.

[5]Carcass-adjusted performance values are based on final BW calculated from hot carcass weight divided by an average dressing percent of 63%; a 4% pencil shrink was applied to initial weights prior; ADG and G:F were calculated over the total days on feed.

Table 3. The influence of supplementing of 10 g vitamin C (VC)·steer[1]·d[-1] for the first 0, 56, 90, or 127 d of the finishing period on plasma ascorbate and glutathione from steers consuming a 0.31 or 0.59% sulfur diet

	HS Con[1]	HS VC56[1]	HS VC90[1]	HS VC127[1]	LS VC56[1]	LS VC90[1]	LS VC127[1]	SEM	1[2]	2[2]	3[2]	4[2]	5[2]	6[2]
Ascorbate														
d 0, μg/L	1,711.7	1,954.8	1,839.2	1,742.7	1,847.7	1,811.1	1,674.4	106.49	0.67	0.28	0.87	0.10	0.23	0.71
d 56, μg/L	1,414.3	1,645.9	1,660.0	1,504.1	1,381.6	1,390.0	1,312.0	127.88	0.06	0.21	0.51	0.17	0.71	0.79
d 90, μg/L	1,301.3	1,129.0	1,254.6	1,322.2	1,260.3	1,229.0	1,272.5	116.80	0.98	0.62	0.83	0.27	0.83	0.79
d 127, μg/L	1,301.0	1,269.5	1,179.6	1,259.7	1,069.8	1,198.1	1,266.1	101.98	0.23	0.49	0.52	0.63	0.09	0.72
Glutathione														
d 0														
tGSH[3], μM/L	7.08	6.85	7.04	6.90	8.61	7.12	7.62	0.540	0.02	0.72	0.79	0.89	0.11	0.05
GSSG[4], μM/L	1.93	1.73	1.66	2.83	1.83	1.83	1.99	0.508	0.60	0.74	0.15	0.10	0.76	0.85
rGSH[5], μM/L	5.16	5.12	5.38	4.91	6.78	5.29	5.57	0.530	0.03	0.96	0.81	0.67	0.05	0.06
GSSG:GSH[6]	0.38	0.34	0.31	0.43	0.28	0.36	0.37	0.065	0.47	0.78	0.60	0.16	0.21	0.56
d 56														
tGSH[3], μM/L	7.86	8.68	8.82	8.95	8.29	8.51	7.88	0.377	0.17	0.01	0.01	0.40	0.33	0.28
GSSG[4], μM/L	1.75	1.05	1.60	1.79	1.79	1.80	1.72	0.213	0.08	0.14	0.63	0.01	0.75	0.82
rGSH[5], μM/L	6.11	7.61	7.21	7.13	6.51	6.72	6.17	0.463	0.10	0.01	0.06	0.07	0.52	0.43
GSSG:GSH[6]	0.38	0.20	0.33	0.33	0.41	0.37	0.39	0.070	0.08	0.12	0.70	0.12	0.80	0.62
d 90														
tGSH[3], μM/L	7.89	7.75	8.06	8.25	7.85	7.79	8.59	0.758	0.83	0.82	0.55	0.72	0.30	0.50
GSSG[4], μM/L	1.70	1.64	1.61	1.60	1.73	1.70	1.76	0.608	0.01	0.13	0.11	0.78	0.67	0.37
rGSH[5], μM/L	6.18	6.08	6.44	6.78	6.23	6.05	6.82	0.774	0.99	0.65	0.38	0.65	0.42	0.47
GSSG:GSH[6]	0.36	0.38	0.36	0.31	0.44	0.41	0.38	0.073	0.18	0.83	0.49	0.55	0.43	0.97

	HS Con[1]	HS VC56[1]	HS VC90[1]	HS VC127[1]	LS VC56[1]	LS VC90[1]	LS VC127[1]	SEM	1[2]	2[2]	3[2]	4[2]	5[2]	6[2]
d 127														
tGSH[3], μM/L	9.73	10.61	9.57	9.81	10.19	10.39	9.71	0.400	0.53	0.44	0.73	0.22	0.32	0.27
GSSG[4], μM/L	2.57	2.19	1.58	2.41	1.02	1.78	1.75	0.671	0.10	0.38	0.58	0.38	0.31	0.50
rGSH[5], μM/L	7.06	8.24	7.86	6.76	9.35	8.52	7.65	0.738	0.05	0.39	0.77	0.08	0.08	0.98
GSSG:GSH[6]	0.40	0.31	0.22	0.44	0.12	0.22	0.30	0.113	0.09	0.42	0.94	0.16	0.20	0.92

[1]Treatments: HS Con: high S control; HS VC56: high S + 10 g VC·steer^{-1}·d^{-1} for the first 56 d of study; HS VC90: high S + 10 g VC·steer^{-1}·d^{-1} for 90 the first 90 d of the study; HS VC127: high S + 10 g VC·steer^{-1}·d^{-1} for the entire finishing period (127 d); LS VC56: low S + 10 g VC·steer^{-1}·d^{-1} for the first 56 d of study; LS VC90: low S + 10 g VC·steer^{-1}·d^{-1} for 90 the first 90 d of the study; LS VC127: low S + 10 g VC·steer^{-1}·d^{-1}for the entire finishing period (127 d).

[2]Contrast statements: 1: high S diets vs. low S diets; 2: control (0 VC) vs. VC supplemented treatments within the high S treatment; 3: linear effect of days of VC supplementation within high S diets; 4: quadratic effect of days of VC supplementation within the high S diets; 5: linear effect of days of VC supplementation within the low S diets; 6: quadratic effect of days of VC supplementation in LS diets.

[3]Total glutathione

[4]Reduced glutathione

[5]Oxidized glutathione

[6]Ratio of oxidized-to-reduced glutathione

Table 4. The influence of supplementing of 10 g vitamin C (VC)·steer[1]·d[-1] for the first 0, 56, 90, or 127 d of the finishing period on d 121 and 122[1] liver mineral concentrations of steers consuming a 0.31 or 0.59% sulfur diett

	HS Con[1]	HS VC56[1]	HS VC90[1]	HS VC127[1]	LS VC56[1]	LS VC90[1]	LS VC127[1]	SEM	1[2]	2[2]	3[2]	4[2]	5[2]	6[2]
Cu, mg/kg	254.1	267.5	290.3	269.9	314.6	292.7	337.8	52.68	0.15	0.63	0.66	0.76	0.65	0.47
Fe, mg/kg	189.4	179.8	229.1	195.6	204.4	252.5	207.2	26.85	0.14	0.60	0.46	0.77	0.96	0.05
Mn, mg/kg	9.9	10.0	9.8	10.9	10.7	9.4	10.0	0.84	0.77	0.68	0.34	0.43	0.46	0.22
Zn, mg/kg	10.6	92.7	104.3	106.0	100.3	98.5	97.7	7.39	0.37	0.31	0.97	0.14	0.74	0.93

[1]Liver biopsies collected on d 121 (n = 24 steers) or d 122 (n = 17 steers)

[2]Treatments: HS Con: high S control; HS VC56: high S + 10 g VC·steer[1]·d[-1] for the first 56 d of study; HS VC90: high S + 10 g VC·steer[1]·d[-1] for 90 the first 90 d of the study; HS VC127: high S + 10 g VC·steer[1]·d[-1] for the entire finishing period (127 d); LS VC56: low S + 10 g VC·steer[1]·d[-1] for the first 56 d of study; LS VC90: low S + 10 g VC·steer[1]·d[-1] for 90 the first 90 d of the study; LS VC127: low S + 10 g VC·steer[1]·d[-1]for the entire finishing period (127 d).

[3]Contrast statements: 1: high S diets vs. low S diets; 2: control (0 VC) vs. VC supplemented treatments within the high S treatment; 3: linear effect of days of VC supplementation within high S diets; 4: quadratic effect of days of VC supplementation within the high S diets; 5: linear effect of days of VC supplementation within the low S diets; 6: quadratic effect of days of VC supplementation in LS diets.

Table 5. The influence of supplementing of 10 g vitamin C (VC)·steer[1]·d[-1] for the first 0, 56, 90, or 127 d of the finishing period on ultrasound measures of steers consuming a 0.31 or 0.59% sulfur diet

	HS Con[1]	HS VC56[1]	HS VC90[1]	HS VC127[1]	LS VC56[1]	LS VC90[1]	LS VC127[1]	SEM	1[2]	2[2]	3[2]	4[2]	5[2]	6[2]
IMF[3], %														
d 56	5.12	4.65	4.21	3.96	4.63	4.98	3.62	0.461	0.84	0.12	0.07	0.98	0.13	0.16
d 90	5.74	5.61	5.37	4.99	5.40	6.17	5.08	0.417	0.69	0.40	0.21	0.66	0.57	0.08
d 127	6.24	5.08	5.29	5.04	5.35	6.51	5.40	0.612	0.39	0.07	0.11	0.45	0.98	0.07
Ribeye area, cm^2														
d 0	46.62	47.01	49.56	47.23	47.54	48.60	48.98	2.338	0.67	0.63	0.68	0.65	0.67	0.90
d 56	68.88	64.85	68.43	68.48	74.23	66.53	73.56	3.228	0.14	0.67	0.95	0.47	0.92	0.09
d 90	82.31	86.09	95.35	86.81	89.87	90.21	88.70	2.677	0.35	0.03	0.05	0.07	0.76	0.79
d 127	92.06	88.32	92.48	94.04	98.01	93.39	95.46	1.885	0.003	0.81	0.24	0.07	0.27	0.08
Back fat, cm														
d 0	0.29	0.23	0.28	0.27	0.28	0.36	0.24	0.044	0.41	0.52	0.81	0.52	0.55	0.06
d 56	0.66	0.58	0.65	0.68	0.78	0.76	0.72	0.083	0.10	0.83	0.80	0.47	0.63	0.96
d 90	0.75	0.67	0.74	0.77	0.94	1.04	0.77	0.078	0.004	0.79	0.80	0.42	0.14	0.08
d 127	1.17	0.95	1.07	1.20	1.26	1.35	1.08	0.128	0.11	0.44	0.81	0.11	0.20	0.16
Rump fat, cm														
d 0	0.36	0.35	0.39	0.29	0.27	0.34	0.38	0.048	0.69	0.70	0.40	0.37	0.12	0.73
d 56	0.87	0.73	0.79	0.80	0.75	0.86	0.82	0.086	0.90	0.37	0.64	0.41	0.61	0.45
d 90	1.08	0.88	1.02	0.97	1.02	1.11	1.08	0.119	0.35	0.33	0.59	0.46	0.74	0.62
d 127	1.18	1.17	1.15	1.28	1.10	1.34	1.32	0.161	0.59	0.90	0.67	0.61	0.25	0.43

[1]Treatments: HS Con: high S control; HS VC56: high S + 10 g VC·steer[1]·d[-1] for the first 56 d of study; HS VC90: high S + 10 g VC·steer[1]·d[-1] for 90 the first 90 d of the study; HS VC127: high S + 10 g VC·steer[1]·d[-1] for the entire finishing period (127 d); LS VC56: low S + 10 g VC·steer[1]·d[-1] for the first 56 d of study; LS VC90: low S + 10 g VC·steer[1]·d[-1] for 90 the first 90 d of the study; LS VC127: low S + 10 g VC·steer[1]·d[-1]for the entire finishing period (127 d).

[2]Contrast statements: 1: high S diets vs. low S diets; 2: control (0 VC) vs. VC supplemented treatments within the high S treatment; 3: linear effect of days of VC supplementation within high S diets; 4: quadratic effect of days of VC supplementation within the high S diets; 5: linear effect of days of VC supplementation within the low S diets; 6: quadratic effect of days of VC supplementation in LS diets.

[3]Intramuscular fat of the ribeye area

Table 6. The influence of supplementing of 10 g VC·steer[1]·d[-1] for 0, 56, 90, or 127 of the finishing period on carcass characteristics and distribution of quality grades and yield grades of steers consuming a 0.31 or 0.59% S diet

	HS Con[1]	HS VC56[1]	HS VC90[1]	HS VC127[1]	LS VC56[1]	LS VC90[1]	LS VC127[1]	SEM	1[2]	2[2]	3[2]	4[2]	5[2]	6[2]
HCW, kg	323.5	310.5	314.7	325.3	337.0	324.7	335.5	10.98	0.12	0.61	0.94	0.31	0.94	0.39
Dressing %	63.8	62.4	62.4	62.1	64.2	62.6	63.3	0.746	0.16	0.05	0.06	0.49	0.30	0.14
Back fat, cm	1.42	1.08	1.27	1.53	1.65	1.72	1.38	0.162	0.05	0.51	0.64	0.08	0.23	0.33
KPH, %	2.17	2.38	2.25	2.25	2.33	2.25	2.50	0.122	0.21	0.30	0.66	0.31	0.23	0.19
REA[3], cm[2]	79.22	79.83	75.78	77.73	79.55	76.67	80.20	2.744	0.71	0.59	0.43	0.94	0.82	0.25
Yield grade	2.33	2.25	2.67	3.00	3.17	3.17	2.50	0.354	0.10	0.38	0.09	0.38	0.11	0.37
1, %	17	0	0	0	0	0	0	--	--	0.10	1.0	1.0	1.0	1.0
2, %	33	75	50	17	17	17	50	--	0.29	0.60	0.50	0.06	0.21	0.52
3, %	50	25	33	67	50	50	50	--	0.50	0.55	0.98	0.32	1.0	1.0
4 and 5, %	0	0	17	17	33	33	0	--	1.0	1.0	0.14	1.0	0.07	0.22
Marbling[4]	607	563	573	548	602	643	602	67.0	0.33	0.49	0.49	0.90	0.99	0.54
QG[5]	4.50	4.00	4.83	3.83	4.67	5.00	4.33	0.639	0.37	0.65	0.60	0.73	0.64	0.45
Prime	17	0	50	0	33	33	17	--	0.13	0.38	1.0	1.0	0.50	0.70
Choice	67	100	50	100	67	67	83	--	0.19	0.13	1.0	1.0	0.50	0.70
Choice^{+}	50	50	0	17	0	25	20	--	1.0	0.18	0.09	0.57	0.56	0.31
Choice^{0}	50	0	67	50	75	75	60	--	0.03	0.30	0.54	0.08	0.62	0.80
Choice^{-}	0	50	33	33	35	0	20	--	1.0	0.07	0.14	0.14	1.0	0.20
Select	17	0	0	0	0	0	0	--	--	0.10	1.0	1.0	1.0	1.0

[1]Treatments: HS Con: high S control; HS VC56: high S + 10 g VC·steer[1]·d[-1] for the first 56 d of study; HS VC90: high S + 10 g VC·steer[1]·d[-1] for 90 the first 90 d of the study; HS VC127: high S + 10 g VC·steer[1]·d[-1] for the entire finishing period (127 d); LS VC56: low S + 10 g VC·steer[1]·d[-1] for the first 56 d of study; LS VC90: low S + 10 g VC·steer[1]·d[-1] for 90 the first 90 d of the study; LS VC127: low S + 10 g VC·steer[1]·d[-1]for the entire finishing period (127 d).

[2]Contrast statements: 1: high S diets vs. low S diets; 2: control (0 VC) vs. VC supplemented treatments within the high S treatment; 3: linear effect of days of VC supplementation within high S diets; 4: quadratic effect of days of VC supplementation within the high S diets; 5: linear effect of days of VC supplementation within the low S diets; 6: quadratic effect of days of VC supplementation in LS diets.

[3]Ribeye area

[4]Marbling scores: modest: 500, moderate: 600, slightly abundant: 700

[5]Quality grade: 3: Choice^{-}, 4: Choice^{0}, 5: Choice^{+}

CHAPTER 9.

GENERAL CONCLUSIONS

Because sulfur (**S**) has been implicated as a contributing factor in the development of oxidative stress, by the depletion of essential antioxidants, the purpose of this research was to characterize the impact that supplementing a rumen-protected vitamin C (**VC**) to finishing steers consuming a high S diet would have on growth performance, antioxidant capacity, carcass characteristics, and meat quality. The studies we designed to address this line of inquiry have generated conflicting results of how supplemental VC and dietary S interacted to influence steer growth performance, carcass characteristics, and meat quality.

This research was developed from a central question to inquire if supplementing a known antioxidant (VC) to cattle consuming a high S diet would help to alleviate some of the negative consequences that excess S in the body is exerting on growth performance, antioxidant capacity, and carcass characteristics. This idea originated from the interrelationship between glutathione (**GSH**) and S removal from the body and the regeneration of oxidized GSH by VC, and the proposal by Truong et al. (2006) that hydrogen sulfide (**H_2S**) is contributing to the development of oxidative stress in hepatic cells by the depletion of GSH stores. Data from the first VC study presented herein confirms this theory, as the ratio of oxidized-to-reduced GSH in calf-fed steers consuming a high S (0.55%) diet for 149 d was 28%, which exceeds the oxidative stress threshold of 10%. Our hypothesis was also asserted that the addition of VC to a 0.55% S diet in calf-fed steers decreased the ratio of oxidized-to reduced liver GSH to 7%, which is below the critical threshold of 10%.

The lag in maximal ruminal sulfate metabolism to H_2S of 10 to 35 days after steers start consuming a high S diet has suggested that steers may be experience some oxidative stress early in the finishing period that may be lingering throughout the finishing and express residual effects postmortem (meat). The staggering of days of VC supplementation for the first 56, 90, or entire finishing period (127 d) was explored in the final VC study, with specific interest on circulating ascorbate and GSH concentrations. Interestingly, steers consuming the high S diet had greater circulating plasma ascorbate on d 56 and subsequently showed greater reduced GSH and less oxidized GSH than their low S counterparts. Furthermore, on d 56 the high S steers consuming VC had greater total and reduced GSH compared to the high S control. Because the steers used in this study were genetically predisposed for greater marbling potential, and are unlikely to represent typical United States feedlot cattle, further examination into the effects of the early supplementation of VC to cattle fed a high S diet is needed.

An intriguing interaction developed across the three studies between supplementing VC in a high S diet and the subsequent plasma ascorbate concentrations and carcass characteristics. In calf-fed steers fed a high S diet, the supplementation of VC for the entire finishing period increased the average plasma ascorbate by approximately 21% (475 and 600 μg/L for the non- and VC-supplemented treatments, respectively) and increased marbling scores 15% from a high Select and low Choice for the un- and VC-supplemented treatments, respectively. Alternately, when plasma ascorbate concentrations exceeded an average of 1,100 and 1,400 μg/L of yearling and calf-fed steers, respectively, fed a high S diet, marbling score was not affected by supplemental VC. This trend for no effect on marbling score when plasma ascorbate was greater than 1,100 μg/L may be suggesting a threshold of plasma

ascorbate to influence marbling potential. Additionally, within the yearling steers, ribeye area linearly increased in a dose dependent manner (0, 5, 10, or 20 g VC per steers per day); however, within the VC supplemented calf-fed steers this trend for greater ribeye area was not observed. Similarly, the threshold of VC theory may also be applicable to postmortem muscle, as the 21% increase in plasma ascorbate in the high S, VC-supplemented calf-fed steers of the first VC study increased the fully autolyzed subunit of calpain-1, a major muscle protease involved in tenderization, by 31% compared to the non-VC supplemented counterparts. Alternately, no difference in calpain-1 autoysis was observed when plasma ascorbate concentrations were greater than 1,100 μg/L in yearling steers consuming a high S diet.

Differences in circulating ascorbate and carcass characteristics may be related to the breed, age, or genetic differences of cattle used in these studies. After consulting the available literature regarding VC supplementation to feedlot cattle, similar patterns for plasma ascorbate and carcass characteristics were identified in Japanese black cattle. It is important to note that while trends may be similar, circulating concentrations of VC are exceedingly greater than those reported herein. Greater ascorbate concentrations may be a factor of differences in physiology and management of Japanese black cattle, as this breed has a greater propensity for marbling and are generally on feed for nearly 3-times the length of typical United States feedlot cattle. Given these differences, Japanese black cattle may not accurately represent the VC needs of feedlot cattle in the United States, as our feedlot cattle are likely to experience higher concentrate and ethanol co-product based diets, receive growth promotants (implants and beta-agonists), and environmental factors (heat, cold, humidity stresses). All of these factors increase the demand for nutrients and possibly

antioxidant stores as cattle continue to grow during the finishing period. Future researchers may wish to focus on further clarifying the requirement of VC by British and Continental breeds of cattle and what subsequent impact United States feedlot management strategies may be having on VC requirements.

Furthermore, the utilization of VC stores for biological processes during the life of feedlot cattle has not been characterized. The downward trend in plasma ascorbate during the initial 90 d of finishing in calf-fed steers may be suggesting that VC is being allocated toward the synthesis of collagens to support growth and development of skeletal, adipose, and muscle tissues. The growth-supporting role of collagen relates to its function in the extracellular matrix, which provides a scaffold for cellular attachment during development and increases turnover to facilitate the expansion of cells during hypertrophy. Vitamin C requirements by calf-fed cattle may be greater than yearlings, as calf-fed cattle likely undergo a more rapid rate of growth during the finishing period since mature frame size, adipose tissue development and hypertrophy, and muscle hypertrophy will be occurring simultaneously. Alternately, yearling cattle enter the feedlot nearer to mature size, the growth that occurs in these cattle will primarily be adipose and muscle hypertrophy. These differences in growth patterns may be indicating differing requirements of VC by calf-fed and yearling steers, thus requiring further examination.